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Artificial Insemination in Livestock Breeding

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INTRODUCTION

Among recent contributions of research to animal husbandry few have been more significant than those resulting from studies of reproductive physiology. Such investigations have elucidated many obscure phenomena of the reproductive processes and have furnished rational bases for certain breeding practices. Concurrently with these developments a great interest has developed in the subject of artificial insemination and much progress has been made in the development of techniques for the application of this method to livestock breeding.

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Artificial insemination refers to the artificial introduction of semen into the genital tract of the female, as contrasted with natural insemination, in which semen is introduced into the female genital tract by the male at the time of copulation. The term "insemination" should not be confused with fertilization and impregnation, for insemination may occur without a resulting fertilization and pregnancy. Fertilization refers to the act of union of the male reproductive cell or spermatozoon with the reproductive cell or egg of the female, as explained by Wilson (*73*).[1] It is possible that fertilization may occur and the resulting fertilized egg may not become implanted (i. e., attached to the mother's uterus), in which case pregnancy is terminated. Artificial insemination has been used extensively as a tool for the improvement of livestock on collectivized farms in the Union of Soviet Socialist Republics by spreading the services of valuable sires over large numbers of females. In the United States no great shortage of good sires has existed, so artificial insemination has not been used to any marked extent, although it offers some possibilities for improvement that can probably be realized in no other way.

HISTORICAL ASPECTS[2]

Although the practical application of artificial insemination is comparatively new, the concept is an old one. There is some evidence that the method was known and perhaps used by Arab horse breeders in the year 700 of the hegira (A. D. 1300), according to LeBon, cited by Heape (*15*). However, the first authentic account of its use in mammals was by an Italian, Spallanzani, who successfully inseminated a bitch about 1780. This result, as described by Heape, was confirmed in 1782 by Pierre Rossi, who went to the special precaution of keeping the bitch used for the experiment under lock and key throughout the entire heat period.

From that time until the latter part of the nineteenth century but few attempts apparently were made to use artificial insemination, although occasional references are made in both medical and veterinary literature to its use as a means of overcoming sterility. Between 1884 and 1896, Heape states further that Sir Everett Millais carefully repeated Spallanzani's experiments, and out of a total of 19 bitches inseminated, 15 conceived. Soon after the beginning of the present century, the practical potentialities of artificial insemination began to be recognized, and in 1907 a Russian physiologist, Iwanoff (*18*), reported the results of a series of successful inseminations in mammals. These experiments were so successful, according to Walton (*65*), that a laboratory was established in 1909 for the purpose of training veterinarians in the technique of artificial insemination.

These studies were interrupted during the World War, but shortly after its close experiments were again undertaken on a large scale and with such practical results that, according to the Russian investigator, Keršin (*19*), more than 6 million cattle and sheep were artificially inseminated in the Soviet Union in 1936. In England, the United States, and elsewhere many investigations also were undertaken after the war. The number of workers in this field has increased

[1] Italic numbers in parentheses refer to Literature Cited, p. 64.

[2] These brief notes on the historical aspects of artificial insemination were taken largely from the papers of Heape (*15*) and Iwanoff (*18*). For a detailed account of the historical aspects of this subject the reader is referred especially to the latter paper.

rapidly, and a vast literature has accumulated concerning this subject. New and better techniques have been devised for the collection of semen, for the handling of the semen from the time of collection to insemination, and for effecting insemination. Hand in hand with these developments, many facts have been established concerning the biology of sperm cells, the secretions of the glands of the reproductive tract of the male, and of estrus and its related phenomena in the female. One of the most recent developments has been the shipping of semen long distances for experimental tests of the value of artificial insemination for long-range use of valuable sires.

USES OF ARTIFICIAL INSEMINATION

Although artificial insemination has been used relatively little in most countries, it has real potentialities for animal improvement. With the development of improved techniques, the percentage of successful inseminations is increasing. The following are some of the uses to which it may be put.

Increasing the Use of Valuable Proved Sires

The reproductive life of sires in most species of livestock is short, and several years at best must elapse before a sire can be tested adequately for such a character as milk production. It is important, therefore, that means be available for making the fullest use of proved sires. Artificial insemination may prove to be an effective means for bringing about such use. By proper coordination arrangements could be made to use semen from valuable sires to effect impregnations of a large number of females in the same and in nearby herds. Furthermore, with improvement in the technique of preservation of semen and the rapid extension of air transportation it is possible that the use of valuable proved sires may be extended to distant herds, as has been recently demonstrated by the experiments of Walton and Prawochenski (*69*) and those of the Bureau of Animal Industry (*50, 63*).

With vigorous sires it should be possible to increase the number of females bred to 1 male several fold. In the Soviet Union, according to Keršin (*19*), as many as 15,000 ewes were inseminated by 1 ram in the breeding season of 1936 and over 1,000 cows by 1 bull. At the Soviet Union breeding centers in 1935, Keršin states that the average percentage of conceptions in ewes was 96.6 and in cows 93.7. He states further that in 1 district all the ewes, 45,000 in number, were inseminated with the semen from 8 rams.

Although such results certainly must represent almost the ultimate in utilization of artificial insemination and results comparable to them are not to be expected under the systems of animal husbandry in practice in most countries, they indicate the use to which valuable proved sires might be put.

Increasing the Percentage of Conceptions

In such species as the horse, where the percentage of conceptions probably does not exceed 50 to 60, according to the English investigators Marshall and Hammond (*35*)[3], the percentage might be in-

[3] Prof. T. A. Ewing, University of Missouri, in a survey of breeding records in Missouri found that of 2,895 mares bred, 1,608 (or 55 percent) settled and 1,323 (45 percent) produced foals.

creased if the mare were served more than once during heat. In the mare ovulation normally occurs 20 to 40 hours before heat ends, and theoretically the best time to mate would be a few hours in advance of ovulation. Accordingly, insemination should be made from 1 to 3 days before the end of heat, and when a mare is still in heat, 3 days after service, it is best to breed her again. Artificial insemination may prove an effective means for bringing about insemination at the period when the chances for conception are greatest and when service by the stallion is not available at the time.

Extending the Period of Usefulness of Valuable Sires

Because of age or crippled condition, a male may be unable to mate normally or, if capable of completing the act, may for similar reasons tire readily and be able to serve only a very limited number of females. Thus artificial insemination may serve as an effective means of extending the period of usefulness of valuable sires which would otherwise have to be slaughtered.

An Effective Aid in Disease Control

When a valuable healthy sire is stood for general use, as in breeding associations, artificial insemination may serve as a means of preventing his exposure to disease and, indirectly, in this way minimize the spread of disease.

Increasing the Use of Sires in Monogamous Species

In species where the males are largely monogamous, artificial insemination offers promise of increasing the use of valuable sires. The development of satisfactory techniques, together with the extensive use of good sires, would be a real boon to breeding progress in such species.

Overcoming Difficulties Due to Difference in Size

If natural service is impossible because of marked difference in size or weight, artificial insemination can be used for effecting conception. This applies especially to cases calling for the breeding of young females to old sires.

Obtaining Species Crosses When Natural Mating is Impracticable

Artificial insemination has been used in a few cases in effecting certain racial and species crosses when, due to differences in size, anatomical structure, or psychological characteristics, natural mating has proved impracticable. In this way it has proved useful as a tool for hybridization experiments.

Effecting Conception in Females That Do Not Conceive After Copulation or That Refuse the Male

In cases of lowered fertility due to temporary disturbances of function in valuable females, artificial insemination has sometimes proved useful in bringing about conception when normal mating has

proved ineffective. It should be used for such purposes with care, however, for some cases of impaired fertility may have an inherent basis. Where there is evidence of a genetic basis for impaired fertility the breeding of such a female should be avoided, or her offspring should be used for purposes other than breeding.

Making Advantageous Use of Young Sires

The use of young sires would be advantageous in certain experimental programs where knowledge of the breeding qualities of a sire is wanted at the earliest possible age. For instance, rams ordinarily are not used for breeding until they are 15 to 20 months old. If by the use of artificial insemination they could be used for breeding as lambs, 1 year would be gained in the age at which the ram was proved. Similarly, young bulls and stallions might be proved at younger ages than would be possible by natural mating.

Use in Breeding Associations and in Large Herds

The greatest promise artificial insemination holds for livestock improvement in the United States is to spread the use of valuable sires, and if used in a large-scale way it should be closely tied into such a program. With the improvement in techniques during the last few years such use of artificial insemination is now feasible. Within recent years a number of cooperative dairy breeding associations have been organized in the United States, the main purpose of which is to obtain for the members of the association the service of proved sires at a reasonable cost. The rules and regulations of unit No. 1 of the organized dairy breeding associations of New Jersey, which was the first association organized to use artificial insemination in the United States, are described by Perry and Bartlett (*48*). General recommendations concerning the organization and the operation of dairy-cattle breeding associations are given by Herman and Ragsdale (*16*).

These associations are under the supervision of a board of directors, or managing committee, elected from the members of the association. Inseminations are carried out by a skilled operator, usually a veterinarian who has had special training in the technique of insemination. In addition to a small membership fee there is a yearly service charge per cow to the members of the association, the amount of this charge differing somewhat in various associations depending on such factors as the number of members and the value of the bull from which service is obtained; but ordinarily it does not exceed $5 per cow. This fee usually includes the insemination of the cow and examination for pregnancy. In most associations this charge does not cover more than two or three services between calvings to the same cow.

For several years artificial insemination has been used to some extent in a few large herds of cattle and on large horse-breeding farms in the United States, but its use in this way thus far has been quite limited. It is probable that such private use of the method will be confined largely to those herds that are large enough to justify the employment of a veterinarian or a technician who can devote his services largely to this task.

Factors of prime importance for the successful use of this method in breeding associations are, (1) a sufficient number of animals in the

association to justify the purchase of superior sires and (2) a sufficient concentration of the females to be inseminated so that the technician can take care of all inseminations on schedule. The operator should be able to make all inseminations within 6 to 8 hours after semen is collected and preferably sooner.

In an effort to improve the status of low-income farmers whom it is assisting, the Farm Security Administration of the United States Department of Agriculture, as of July 1, 1939, had organized more than 3,500 breeding groups or associations having to do with dairy cattle and various other forms of livestock. In a few of these associations artificial insemination is now being used, and steps are under way to use it in many more of them. Because of their limited resources most of these farmers cannot afford to buy first-class sires, so the use of artificial insemination should prove of great value to this program.

To facilitate organization of such associations the Department of Agriculture has recently prepared a suggested constitution and bylaws for livestock breeding associations, which may serve as a model for any associations that want to undertake the use of artificial insemination for the improvement of their livestock.[4] A rather complete plan of work for promoting and organizing artificial breeding circuits has recently been issued in mimeograph form by the New York State Extension Service.

LIMITATIONS OF ARTIFICIAL INSEMINATION

Although artificial insemination is a promising procedure for the breeder, it must be emphasized that it can be used successfully and with safety only by skilled persons, such as veterinarians specially trained. It is necessary to have knowledge of the structure of the reproductive organs and of the procedures to be followed in collecting the semen and introducing it into the genital tract of the female. Sperm are very delicate and must be handled with great care if they are to retain their full vitality, which is necessary for the highest percentage of successful impregnations. Care needs to be taken also to avoid injury to the reproductive system of both male and female and to prevent the possible spread of disease

It should be emphasized that relatively little research has been done on artificial insemination of some species of animals. Until further information is available, the practice of artificial insemination in such cases will probably not be completely satisfactory. For it to be most successful, satisfactory methods for collection of semen and for insemination must be available; and the stage of the estrual cycle must be known so that insemination can be made at the proper time with respect to ovulation.

PROCEDURES INVOLVED IN ARTIFICIAL INSEMINATION

Two distinct operations are involved in the process of artificial insemination. The first is the collection of the semen from the male and the second the insemination of the female. However, if artificial

[4] Mimeographed copies of this constitution and bylaws may be obtained by writing to the Bureaus of Animal Industry or Dairy Industry or to the Farm Security Administration of the U. S. Department of Agriculture.

insemination is to be employed to any great extent in a breeding program other factors must be considered if it is to be highly successful. These additional factors involve such things as the handling and examination of the semen after collection, the care and management of the male, the frequency of use of the male, and the proper timing of insemination in relation to estrus and ovulation.

This section discusses the more general problems encountered in the use of artificial insemination, reserving a discussion of the details of technique of collection and insemination for a later section since these vary somewhat from species to species.

Various methods of collecting semen have been devised, but certain of these are unsatisfactory and are gradually being replaced by newer and more satisfactory techniques. However, since some of the older methods are still in rather general use and other methods are useful under certain conditions, most methods that have been used for the collection of semen are described, and a discussion of the advantages and limitations of each is presented.

Collection of Semen by Approved Methods

by means of the artificial vagina

During the last few years the artificial vagina has come into extensive use for the collection of semen, especially in the Soviet Union,

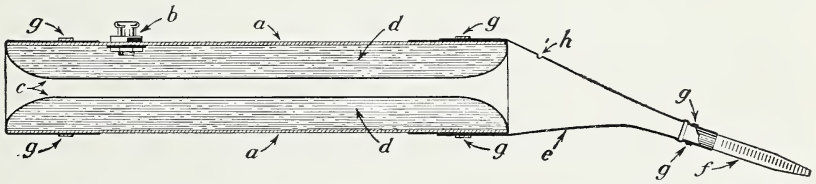

Figure 1.—Longitudinal section of artificial vagina for cattle; *a*, Outer rubber casing 16 by 2¾ inches; *b*, brass valve for introducing warm water; *c*, thin-rubber inner tube; *d*, space for water; *e*, thin-rubber collecting tube tapering at one end; *f*, glass test tube or vial; *g*, rubber band; *h*, air vent to prevent ballooning.

where it has been used on a large scale. It is a method that is equally valuable for field and for laboratory use. The apparatus consists essentially of a thin rubber tube kept warm by a surrounding air or water jacket. It is composed of an outer cylinder of heavy rubber casing, glass, ebonite, or metal with an inner rubber sleeve which is attached to the outer cylinder in such a way that a watertight space is created between the inner and outer walls (fig. 1). One end of the inner tube is open; the tube at the other end is tapered to fit tightly over a graduated test tube or a special-type graduated glass container[5] in which the semen is collected at the time of ejaculation. One or more small valves may be provided in the outer wall (*a*) for the purpose of introducing warm water into the space between the inner and outer surfaces (*a* and *c*). A small vent (*h*) is provided on the upper side of the tapering tube for the escape of air to prevent ballooning. The container into which the semen is ejaculated is made in some models with an inner flange which holds the semen within the container. In

[5] An ungraduated test tube or container may be used for the collection of the semen if it is unnecessary to measure the volume.

the model in which the graduated test tube is used for collection of the semen the same effect is obtained as the tube is pendant at the end of the rubber tube so that the semen flows down and is collected in the test tube. The volume of semen in the ejaculate may be determined readily from the graduated container.

In general very little difficulty is encountered in using the artificial vagina. The ejaculate is collected in the receptacle in an almost sterile condition. In using the artificial vagina care must be taken to have the water at the right temperature at the time of collection. If it is either too hot or too cold the male may refuse service. A temperature of 41° C. is near optimum for most species. The size of the inner aperture and hence the pressure on the penis is controlled largely by the quantity of water added. The pressure may be regulated also by blowing air through one of the valves after the water is added. The optimum size for the opening will vary somewhat from male to male, but with experience an operator will be able to regulate this so that little trouble will be encountered in obtaining ejaculates, provided the temperature is right and the inner sleeve is well lubricated.[6] Most males will work readily with the artificial vagina.

The advantages of artificial vaginas are: (1) Practically the entire ejaculate is collected, (2) the semen is free from extraneous secretions, (3) because of its near-sterile condition the danger of spread of disease from that source is minimized, (4) the volume of the ejaculate is easily measured and its characteristics easily determined because of its freedom from extraneous substances, (5) the viability of the spermatozoa in semen so obtained is higher than when other methods of collection are used, and (6) no female is needed for collection whenever a dummy is used successfully. The disadvantages of the method are: (1) Difficulty is occasionally encountered in getting males to serve the artificial vagina, (2) the apparatus is somewhat costly, and (3) more care must be taken if it is to be used successfully than is needed with some of the other methods.

FROM THE VAGINA

In this method semen is collected from the anterior end of the vagina following service. For collection by this method it is not necessary in most species to have a female in heat for most males may be easily trained to mount a female out of heat. The semen may be recovered from the vagina by a suitable syringe, with or without the aid of a speculum, or a vaginal spoon may be used conveniently (fig. 2). The vaginal spoon is especially useful in obtaining a sample of semen for examination.

This method of semen collection has a number of advantages: (1) It is exceedingly simple, and the outlay for apparatus is very small, (2) mating and ejaculation are entirely normal, and (3) there is little need for laboratory preparation. On the other hand, there are certain disadvantages: (1) The quantity of semen collected is usually small as not all of it can be withdrawn from the vagina, (2) it is mixed with the vaginal secretions and these are very often detrimental to spermatozoa especially when it is necessary to store semen for considerable periods, (3) there is some danger of injury to the reproductive tract of the

[6] A good lubricant can be made by mixing thoroughly 6 gm. of powdered gum tragacanth with 10 cc. of glycerin. Add to this mixture, stirring continuously, 100 cc. of water. Keep in refrigerator to prevent molding. This lubricant is recommended for general use in artificial insemination.

female at collection or to the penis of the male when the female is not in estrus, and (4) there is a greater chance for the spread of disease, should the female be affected.

This technique has been used to some extent in combating mechanical sterility in the mare. The semen is first withdrawn from the vagina, then reinjected into the cervix or even directly into the uterus. It is a valuable technique to supplement natural service when the

FIGURE 2.—Vaginal spoon (Nils Lagerlöf type) used for the collection of semen from heifers and cows. The spoon should be about 1 inch wide, 5 inches long, and the bowl ½ inch deep; the entire length, including handle, is about 30 inches. Preferably it should be made of ebonite.

stallion serves short and the semen is deposited on the floor of the vagina.

THE BREEDER'S BAG

This method is suitable only for the stallion and jack and is described under the practice of artificial insemination in horses and asses. Its use permits the collection of whole ejaculates in a practically uncontaminated condition.

THE INDUCTION OF EJACULATION BY MECHANICAL MANIPULATION

This method has been used successfully only in cattle, in dogs, and in fowls. The technique of collection from the bull, as developed by Miller and Evans (*36*), of the United States Department of Agriculture, consists in massage of the accessory genital organs, the greatest volume of semen being collected by massaging the ampullae of the ductuli deferentia. To do this the arm must be inserted into the rectum, the accessory glands must be located and then gently squeezed. If this is properly done, the semen contained in the ampullae is forced out and is collected by means of a funnel and test tube.

This method has several disadvantages, chief of which is the fact that considerable skill and knowledge of the anatomy of the bull are required of the operator. Other difficulties that are encountered are: (1) Some bulls cannot be made to relax sufficiently for the operator to press out the secretions, (2) in many cases it is difficult to obtain the semen entirely free of urine although the skilled operator usually can obtain semen without urinary contamination, (3) considerable care must be used in collection, or the semen becomes contaminated by coming in contact with the prepuce or by the secretions of the preputial glands, and (4) some operators have reported inability to obtain large quantities of semen by this means. If reasonable care and gentleness are exercised no apparent injury to the bull results from the massage, and collections may be made at frequent intervals, even daily. This method requires great skill but has been used in some cases in insemination practice in private herds as described by Kingman (*20*) and others.

The advantages of the method are: (1) Semen can be obtained from valuable sires that are unable to mount a cow because of injuries or weakened hind legs, and (2) there is probably less danger of the spread of infections such as trichomoniasis, than by any other method, except perhaps the artificial vagina.

To obtain semen from the dog, the base of the penis is massaged with the hand. This induces erection and ejaculation. Dogs may be used for collection of semen for long periods without injury to their health. This method of obtaining semen has a number of advantages: (1) No female is required; (2) the technique is simple, and no special equipment is needed; and (3) the semen is obtained in a relatively pure condition.

A very satisfactory technique for the collection of semen from the domestic fowl and turkey has been developed by two Department biologists, Burrows and Quinn (*7*, *8*). With this method the bird is slightly stimulated until the copulatory organ is protruded, the base of this organ then being gripped with the thumb and forefinger and the semen contained in the bulbous ducts milked out. At one collection quantities of semen varying from 0.1 cc. to as much as 4 cc. in a few instances may be obtained. As will subsequently be brought out, males vary greatly in their response.

THE INDUCTION OF EJACULATION BY ELECTRICAL STIMULATION

This method for the collection of semen, which is described fully by an Australian, Gunn (*12*), is essentially a laboratory method and one that is not suitable for use with all species of animals. Gunn has applied it very successfully in the collection of semen from rams and reports that repeated collections may be made from the same ram without harmful results. Gunn's findings have been confirmed by several workers. Successful collections also have been reported with foxes, rabbits, and guinea pigs, but the method has not been so successful with dogs. The quality of the semen obtained was normal, and no deleterious effect was observed on viability or motility of the spermatozoa.

The method has a number of disadvantages: (1) The apparatus needed is expensive, (2) great care must be exercised to control the degree of shock applied, (3) the semen is sometimes contaminated with urine, and (4) considerable skill is required of the operator if successful collections are to be made. The chief advantages are: (1) The semen is obtained in a near-sterile condition, and (2) the method is effective for use on valuable rams that cannot copulate normally because of injury, in rams that have lost their sexual desire, or in rams too young to have learned to copulate.

The Collection of Semen by Obsolete and Other Methods

THE SPONGE METHOD

The sponge method is one of the oldest methods of collection but has largely been supplanted by other methods. It consists of introducing a soft rubber sponge which has been treated with physiological saline solution or some type of sperm-diluting fluid into the anterior end of the vagina. When the sponge is in place coitus is allowed,

after which the sponge is withdrawn from the vagina, and the semen is removed by squeezing. The method has a number of disadvantages, among which are: (1) A large number of sperm are injured when the sponge is squeezed in order to collect the semen, (2) the semen is not obtained in pure condition but is mixed with vaginal secretions which are absorbed by the sponge, (3) not all of the semen is collected by the sponge, and (4) there is the chance of spread of disease.

Some of the objectionable features can be minimized by proper manipulation of the sponge. For instance, if the semen is squeezed from the surface of the sponge where it was deposited at ejaculation, more undamaged sperm are recovered. The method, however, is not to be recommended if other means of collection are available. Walton, (*65*) has pointed out that in addition to the loss of sperm from injury and inability to squeeze all the semen from the sponge, there is a loss of 40 to 50 percent in the motility of spermatozoa so obtained and that the time of survival of motility of sperm collected from four bulls by the sponge method was only about one-half as long as in semen collected by other methods.

THE "SPERM COLLECTOR"

The "sperm collector," so-called, is suitable for use only with sheep and cattle. The apparatus consists of an elongated thin rubber tube blind at one end and expanded on its open end into an elliptical shield by means of attachment to a flexible metal ring. It is held in place in some models by an inflatable rubber ring and in others by means of a small flexible metal spring which is molded into the rubber. The sperm collector has a number of objectionable features: (1) It is somewhat difficult to introduce into the vagina, (2) in some cases the penis, especially of the bull, is inserted outside the outer ring or shield, in which case no semen is collected, (3) the penis is sometimes irritated by the outer ring of the apparatus, (4) it is difficult to get some males to serve if the sperm collector is used, (5) it is difficult to keep in place, and (6) the apparatus is perishable and somewhat costly. Its advantage lies in the fact that the semen is collected in an undiluted state and the viability of the spermatozoa in semen so collected is high. Most investigators have found the apparatus rather unsatisfactory, and it probably will be supplanted almost entirely by the artificial vagina.

PAN COLLECTION AFTER COITUS

A method that has been used to some extent for the collection of semen in horses is to catch in a pan the part of the ejaculate that is passed following withdrawal after natural service. While some successful inseminations may be obtained from semen collected in this manner, the method is not to be recommended, because the volume of semen collected is usually small and the sperm concentration in this part of the ejaculate varies from 0 to 5 percent of the concentration observed in the whole ejaculate.

THE FISTULA METHOD

The fistula method is sometimes useful for the collection of semen samples, but it is suitable only for laboratory purposes. It involves surgical interference and the introduction of a tube into the urethra. Semen is collected at the time of copulation.

EXAMINATION OF SEMEN

Great variation exists in the quantity and quality of the semen obtained from different males and also in the quantity and quality obtained from the same male at different times. Consequently the semen of any male should be carefully examined before it is used for insemination. The points to be considered in this examination are: (1) The volume of the semen, (2) the color and cloudiness, (3) the consistency, (4) the motility of the sperm, (5) the number of normal sperm per cubic millimeter of semen, (6) freedom from bacteria, parasites, or cells that would indicate a pathological condition of the genital organs of the male from which the sample is obtained, (7) the hydrogen-ion concentration of the semen (table 3), and (8) the respiration rate of the sperm. In males intended for extensive use it is desirable that semen examinations be complete, for it has been shown by Phillips (*49*) and Lagerlöf (*28*) that there is a relationship between certain of the above characteristics and fertility. In large-scale breeding programs sires should be subjected to such examinations prior to and at intervals throughout the breeding season. In addition, Williams and Savage (*72*) state that a competent clinical examination of the genitals of breeding animals is necessary if breeding losses are to be prevented and controlled.

As the number of inseminations that can be made from any one male is dependent upon the volume of semen produced, it is important to use males producing large quantities of semen containing large numbers of active, viable spermatozoa. Volume of semen may be measured in a graduated test tube, bottle, or pipette. Although a large volume of semen does not necessarily mean a high sperm count as shown by McKenzie and Berliner (*31*), there is a reasonably high correlation for most animals between the total quantity of semen in the ejaculate and the number of spermatozoa contained therein, provided, of course, that the ejaculate is complete. Measurement of the volume of semen production for any given sire should not be made upon one ejaculate but upon the total ejaculate produced in a given interval of time upon several different occasions.

Both volume of semen and number and quality of spermatozoa are reduced during certain periods of the year in sheep, according to McKenzie and Berliner (*31*), and this is likely true for all species of animals, especially those having a more or less limited natural breeding season.

The color and consistency of semen give some clue as to its quality. These characteristics depend on the species concerned.[7] A yellowish color may indicate the presence of pus or urine, which may often be detected also by smell. A pinkish or reddish color indicates an admixture of fresh blood, while a deeper red or brownish coloration probably indicates the presence of degenerating blood and tissues. A greenish coloration may indicate purulent degeneration. Such abnormal coloration of the semen may indicate abnormal conditions in the genital organs of the male, or possibly of the female in case the sample was collected directly from the vagina. A sire from which abnormal semen is collected should not be used for insemination or breeding purposes until the cause of the abnormalities has been

[7] See section on The Practice of Artificial Insemination for the characteristics of normal semen for the different species.

established and removed. If semen is collected from the vagina, care should be used to see that the female is healthy. Because of admixture with vaginal secretions semen so collected will have a somewhat different appearance than that collected in other ways.

Siebenga (*59*), a Dutch investigator, found the cloud formation in bull semen a dependable index of quality. When the swirling cloudiness was marked he obtained 84 percent of conceptions in a total of 206 inseminations; when there was little cloudiness, 55 percent in 47 inseminations; when cloudiness was slight, 25 percent in 20 cases; and when there was no cloudiness, only 1 pregnancy in 19 inseminations, or 5.3 percent.

Determination of motility, number of spermatozoa per cubic millimeter of ejaculate, abnormalities of the sperm, and examination for pathological cells are made with the microscope. For routine examinations magnifications of 300 to 400 diameters are most satisfactory. Samples for examination should be taken immediately after collection, and care should be exercised to see that the sample is representative of the whole ejaculate. It has been pointed out by McKenzie, Miller, and Bauguess (*32*), Kingman (*20*), and others that certain portions of the ejaculate may be low in sperm concentration. This is especially true of the stallion, jack, boar, and dog in which the secretions of the accessory reproductive glands make up a large part of the ejaculate.

Examination for motility is best made by means of the hanging-drop technique. This is accomplished by placing a well-mixed drop of semen on a cover slip and inverting it on a hollow-ground slide. In this examination care must be taken to prevent any great drop in temperature of the semen for movement is greatly retarded by cooling and ceases altogether at lower temperatures. To accomplish this, the examination should be made in a warm room or by providing some means for keeping the sample at a constant temperature of 38° to 40° C., such as a slide incubator, a heating pad, or hot-water bottle. Care must be used, however, not to have the temperature too high, as Walton (*65*) has shown that sperm are killed at 46°. In case hollow-ground slides are not available, a fairly satisfactory examination for motility may be made by placing a drop of semen on a cover slip, inverting this on a glass slide, and having each end of the cover slip supported by other cover slips, thus providing space for the semen drop without pressure on the sperm.

Various types of motility are observed. According to Walton (*65*), three types of motion may be recognized: (1) A progressive motion in which the spermatozoon usually moves in a straight line (for mammals), (2) a rotary motion in which the movement is along the edge of a circle whose diameter is usually not longer than the spermatozoon, and (3) an oscillatory motion in which the movements consist of convulsive motions without change of place. Walton considers that the sperm must possess progressive motion if it is to meet and fertilize the ovum.

No very satisfactory way of classifying motility has been devised although several methods have been used, as shown by Roemmele (*57*), Moore (*41*), Gunn (*12*), and others. According to Walton (*65*) Russian workers have devised a rather simple system for grading motility which is adequate for practical purposes. It is based upon a discrimination in various types of motility and the proportion of sperms exhibiting the respective types. Semen in which nearly all the sperma-

tozoa exhibit energetic progressive motion are graded 5, the highest mark; semen in which most of the sperm have progressive motion are graded 4; samples in which there are about equal proportions of sperm exhibiting progressive motion and oscillatory motion or are immotile are graded only 3; samples with oscillatory motion and large numbers immotile are graded 2; those that are immotile are graded 1; and if no sperm are present, the grade is 0. Gunn (*12*) has proposed the following classification: Very actively motile, actively motile, motile, sluggishly motile, and nonmotile. These are given arbitrary numerical values of 100, 75, 50, 25, and 0, respectively, from which a percentage motility factor is derived by multiplying the arbitrary numerical values for motility by the percentage of spermatozoa showing such motility and dividing by 100. This factor indicates the effectiveness of motility of any given sample.

The presence of large numbers of abnormal spermatozoa in the ejaculate of a male may indicate spermatic derangement and a reduction in fertility. Consequently the examination of the semen of potential sires is a matter of practical importance in order that males of low fertility owing to a high percentage of abnormal spermatozoa may be detected.

Various types of abnormalities, which have been described by the Swedish investigator Lagerlöf (*27*) and others, are observed. These include abnormal heads, such as tapering heads, shrunken heads, large heads, and twin heads; abnormalities of the neck region, such as tailless heads and broken necks; and abnormalities of the middle piece, including such derangements as enlargements, shortness of the middle piece and tail, filiform middle pieces, and broken and bent middle pieces. Abnormalities of the tail include such conditions as coiled tails, twin tails, broken tails, and tails stuck together.

Although an exact correlation between the percentage of abnormal cells and fertility has not been and perhaps cannot be established, various authors have shown a relationship between abnormalities and low fertility. Thus, Moench and Holt (*40*) have pointed out that in men of good fertility the percentage of abnormal sperm heads does not exceed 20 percent, whereas men having more than 25 percent of abnormalities are sterile. Abnormalities of the semen of normal bulls do not exceed 17 percent, according to Williams and Savage (*72*), and according to McKenzie and Phillips (*34*) the figure for normal rams does not exceed 15 percent. In boars, also, a similar relationship exists. McKenzie and Phillips (*33*) reported that semen from fertile boars does not contain more than 62 to 104 abnormalities per 1,000 spermatozoa, whereas boars whose semen contained from 146 to 501 abnormalities for 1,000 sperms were siring small litters containing mummies and weak pigs. Phillips (*49*) has made a further study of this problem in the boar. Although these studies did not justify the establishment of definite limits regarding the number of abnormal spermatozoa in the semen of boars of various degrees of fertility, they did indicate a direct relation between the presence of abnormal spermatozoa and low fertility.

When examination is made for abnormalities, due allowance must be made for such factors as season of year, previous sexual activity, and method of collection before final rating is made of any given sire. It has been shown clearly by McKenzie and Berliner (*31*) that for rams the number of abnormal spermatozoa is much higher out of the

regular breeding season, some rams actually showing as many as 700 to 900 abnormal sperms per 1,000 during June (fig. 3).

The manner of collection also influences the abnormality count as well as the total volume of semen collected. If a sponge is used for collection, the percentage of abnormal sperms will be high, because many spermatozoa will be damaged when they are squeezed out of the sponge. If collection is made directly from the vagina, care must be exercised to see that sperm from a previous service are not collected with the fresh ejaculate. The life of spermatozoa is relatively short in the vagina, according to Warbritton et al. (*70*), and if collection is made from a female that has been served less than about 30 hours

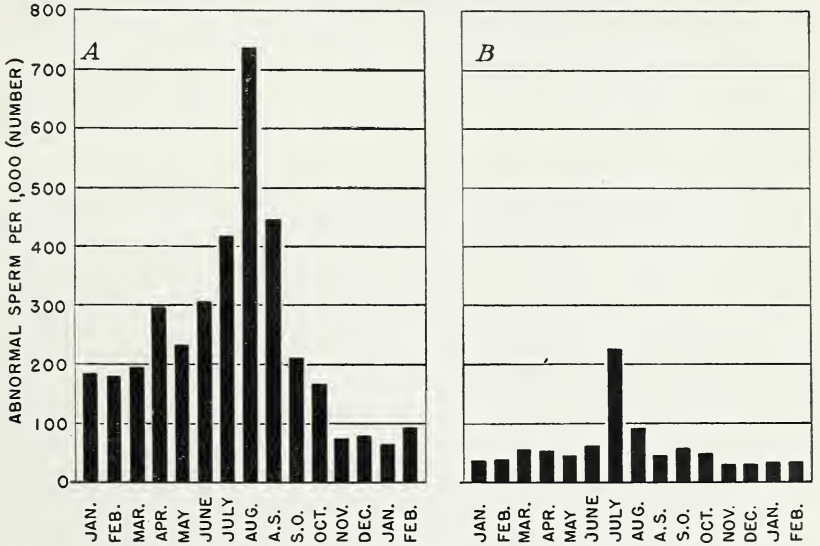

FIGURE 3.—The influence of season upon the production of abnormal sperm in sheep, January 1935 to February 1936. The counts in each period (January to February, inclusive) were made over a 28-day interval, this resulting in considerable overlapping during the months of August to October: *A*, Shropshires; *B*, Hampshires. (Adapted from Missouri Agricultural Experiment Station Research Bulletin 265.)

earlier, sufficient degenerate sperm from the earlier service may be collected to increase greatly the abnormal count of the male under test.

The state of breeding activity of the ram may also influence the abnormal sperm content of the semen. Some investigators, including Walton (*65*), of England, Webster (*71*), of Australia, McKenzie and Phillips (*34*), of the United States, and others have observed that there were many more abnormal sperm in the first ejaculate after an extended period of sexual rest. Other writers, Gunn (*12*), of Australia, and Rodolfo (*55*), of the Philippine Islands, have failed to confirm these findings. However, since spermatozoa of males that do not copulate frequently collect in the epididymis and vas deferens and undergo degeneration according to Simeone and Young (*60*), it would seem that examinations should be made only on semen collected after a male has copulated at least once and preferably several times.

Examination for abnormalities is made by placing a small drop of well-mixed semen on a microscope slide and spreading this by drawing

the edge of a cover slip in the same manner that blood smears are made. Another method consists in gently dropping another slide onto the semen and then separating the two slides. The slide is then labeled and set aside in a clean, dust-free place to dry, after which it is stained for examination.

The staining may be carried out by any of the following methods:

1. Dried semen smears may be stained satisfactorily with 0.5-percent alcohol solution of gentian violet for 3 minutes.

2. The slide may be air-dried, put in a saturated solution of chlorazene for 5 to 10 minutes, rinsed in distilled water, fixed in 10-percent formalin for 3 to 5 minutes, rinsed in distilled water, stained with Ziehl's carbol-fuchsin for 2 minutes, washed gently in running tap water, and dried.

3. Lagerlöf (*27*) gives the following instructions for demonstrating protoplasmic drops in bull semen: Immediately after collecting the semen place 2 drops of semen on a slide and mix with the semen 3 drops of opal blue (Bresslau). See that the opal blue has been heated till it steams and that it is then filtered and cooled to near room temperature, this preparation to be done immediately preceding the staining of the semen. After the stain and the semen have been mixed, spread and allow to air-dry. Examination can be made with the slide wet or dry.

The number of sperm per given unit of semen may be most accurately determined by means of a hemocytometer. For this examination the semen is diluted 100 or more times with 0.9-percent saline solution by means of a special pipette. After dilution and thorough mixing, a drop of the solution is placed on the counting chamber, and the spermatozoa are counted for a definite number of squares on the counting chamber. When this number is obtained, the number per cubic millimeter of semen may be calculated.

The apparatus used for this examination must be clean, dry, and free from grease, and the semen should be well-mixed before a sample is taken. After the semen and saline solution are drawn up in the pipette, they should be well shaken and the first three or four drops of the mixture discarded, since these drops are not likely to represent a well-mixed sample of semen and saline. The drop placed on the counting chamber should not be too large, and the layer of fluid should be of an even thickness under the cover slip before the counts are made. Only spermatozoa whose heads lie within the squares should be counted. Of those heads that lie upon lines of demarcation between squares, only those on two sides of the squares are counted; heads lying on the lines demarking the other two sides of the square are not counted.

An adequate technique for counting bull and ram sperm [5] is carried out as follows, all operations to be conducted at room temperature:

PROCEDURE

1. Stir sample well.
2. Take 0.1 cc. with 0.2-cc. pipette graduated in 0.1 cc. units.
3. Add 9.9 cc. of 0.9-percent sodium chloride from a burette (thus securing one one-hundreth dilution of the semen).
4. Take 0.1 cc. of the diluted semen of step 3.[5]
5. Add 9.9 cc. of 0.9-percent sodium chloride (thus securing one ten-thousandth dilution).
6. Take up in pipette with rubber bulb, after agitating several times by pressing and releasing bulb; waste a few drops; then with equal pressure put a drop on the counting slide.
7. Wait 10 minutes for sperm to settle.
8. Make three counting slides.
9. Count 16 to 64 squares in each slide, the number of squares counted depending on whether or not the sample is concentrated.

[5] Steps 4 and 5 are omitted in preparing horse and boar semen for counting, a dilution of 1:100 being sufficient.

CALCULATION

0.2 mm. (marked on slide) times $\frac{1}{16}$ sq. mm. $=\frac{1}{80}$ cu. mm.

Number of sperm per square $=\frac{a}{b}$ (a = number of sperm; b = number of squares counted)

Number of sperm per cubic millimeter $=\frac{a}{b\times\frac{1}{80}}=\frac{a\times 80}{b}$

Or, number of sperm per cubic centimeter (or density) $=\frac{(a\times 80)}{b}\times 1{,}000$

Multiply by 10,000 to get the number of sperm per cubic centimeter, because dilution is 1 to 10,000.

For example, 21 sperm in 16 squares
27 sperm in 16 squares
24 sperm in 16 squares

72 48

Density $=\frac{72\times 80}{48}\times 10{,}000=1{,}200{,}000$ sperm per cubic millimeter, or 1,200,000,000 sperm per cubic centimeter.

Semen examinations should also include such items as bacteria, protozoa, and cells which might indicate a pathologic condition of the male. In case the semen is collected from the vagina of a female, the operator must be sure that the female used for this purpose is healthy, and even then it is best that an examination of the vaginal secretions be made before she is used for collection. It must be remembered that the vagina is never free from fecal contamination, so it is probable that the bacterial count of the vaginal fluid will be high. To lower this count as much as possible, it may be best first to flush the vagina well with physiological saline or with some type of semen diluting fluid.

The presence of excessive numbers of bacteria or masses of bacteria and cells or of cell debris in a semen sample may be indicative of a diseased condition. Such males should not be used until the cause for the abnormal bacteria and cell content is determined or until subsequent examinations prove that the abnormal condition has cleared up. In making such an examination of cattle the operator should look for trichomonads.

The hydrogen-ion concentration can best be determined by the use of an electric potentiometer with calomel cell and quinhydrone. The hydrogen-ion content of normal semen is indicated for each of several species in table 3 (p. 36).

Recently attention has been given to what promises to be a more critical method of evaluating semen quality, namely that of measuring the respiration of spermatozoa. Walton and Edwards (*68*) have correlated their findings on sperm respiration with breeding performance in bulls and find it the most reliable index of fertility (table 1).

TABLE 1.—*The relation between breeding performance and respiration rate of sperm as observed by the English investigators, Walton and Edwards (68)*

Number of bulls	Services per conception	Average initial respiration rate	Average respiration rate after 2 hours
	Number		
3	1–1.9	0.286	0.058
6	2–2.9	.187	.035
4	3–6.9	.127	.012

HANDLING AND TRANSPORTING SEMEN AFTER COLLECTION

Protection From Temperature Shock and From Air

If inseminations are not to be made immediately after collection of semen, certain precautions must be taken to keep the spermatozoa in a high state of viability if the largest percentage of conceptions is to ensue. Temperature shocks should be avoided, and it is best to protect the sample as much as possible from contact with air in order to lower the metabolic activity of the spermatozoa, thereby conserving their vitality. In addition, contact with water and harmful chemical agents must be scrupulously avoided.

The manner of handling the semen will depend to a large extent on the interval to elapse between collection and insemination. If

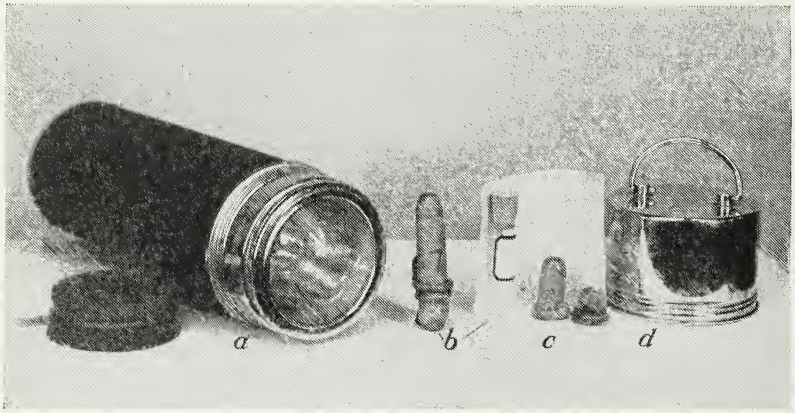

Figure 4.—Equipment for transporting semen: *a*, Wide-mouthed, quart vacuum bottle with cork at extreme left; *b*, small glass vials for holding the semen; *c*, paper and two thumbstalls for wrapping the vial (vial at left is shown labeled and wrapped); *d*, top for the vacuum bottle.

inseminations are to be made within 2 hours after collection, the precautions to be taken are relatively simple. In such cases it is sufficient to place the semen in a small stoppered vial which has been thoroughly cleansed and dried. The vial may then be left at room temperature until the semen is used, but it should be kept in a closed container or in a dark place, and under no conditions should it be exposed to direct sunlight. If the semen is to be kept for longer periods, additional precautions must be taken.

Immediately after collection the semen should be placed in a clean vial, covered with a layer of high-grade neutral paraffin oil up to the cork, thus eliminating any air space in the vial. The vial should then be wrapped in two thicknesses of paper and set in the refrigerator, or the paper-wrapped vial may be covered with two rubber thumbstalls and dropped in a vacuum flask containing water at from 3° to 8° C. This provides for a gradual cooling of the sample and maintenance at a temperature of from 3° to 8°. The equipment needed is shown in figure 4. If one wishes to avoid the use of paraffin oil he can do so by

using paraffin wax plugs pressed into the vials down to the semen. Two or three drops of melted paraffin is poured over the wax plug to seal the vial.

The above-described technique has been successfully used with bull, ram, and goat semen, but different storage methods are needed for the more watery types of semen such as those of the horse and boar. Walton and Prawochenski (*69*) have satisfactorily stored horse semen 24 hours by first separating out the glairy, viscous portion of the ejaculate and then centrifuging the rest and storing the centrifuged sperm at 0° to 3° C. At the Missouri station boar semen has been successfully stored for 56 hours by removing the gelatinous lumps and then adding an equal volume of the swine diluter described by Milovanov (*37* and *38*) and given in table 5.[9] Storage was at 10° to 12°. Berliner and coworkers (*5*) emphasize the need of gradually changing the temperature of equine semen.

Preparation of Semen for Long-Distance Shipments

When semen of the bull, ram, or goat is to be shipped a long distance, more elaborate precautions are necessary (fig. 4). The vial containing the semen is wrapped in a layer of cotton, after which it should be placed in a screw-top, watertight glass vial, which is in turn well wrapped in cotton which is held in place by rubber bands. The package is then placed in a quart vacuum bottle and tightly packed with chipped ice but no salt. To avoid danger of breakage, it is best to place a layer of cotton in the bottom of the vacuum bottle before packing with ice and to add a layer of cotton before stoppering. If properly packed in this manner, the semen may be kept at a temperature below 10° C. for about 30 hours.

Upon removal of the semen from the refrigerator or the vacuum bottle, it is best to raise its temperature gradually before inseminations are made. This may be done by allowing the vial to stand at room temperature for from 30 minutes to an hour, after which the vial may be set in tepid water (33° to 35° C.) for a few minutes. Care must be taken when artificial means are used for warming the semen to avoid temperatures above 35°.

In the last few years considerable interest has developed in the transportation of semen for insemination in distant herds. Although the practical difficulties to be overcome probably preclude any extensive use of this technique, recent experiments have demonstrated possibilities for its use. Thus, Walton (*64*) reported successful inseminations with rabbit semen shipped from Cambridge, England, to Edinburgh, Scotland, and Walton and Prawochenski (*69*) shipped ram semen from England to Poland and obtained successful impregnations as late as 27 hours after collection. Investigators in the Bureau of Animal Industry, Terrill and Gildow (*63*) and Phillips, Schott, and Gildow (*50*), have shipped ram semen from two of its experiment stations, located at Beltsville, Md., and at Dubois, Idaho, to the Idaho Agricultural Experiment Station, at Moscow, Idaho, and obtained eight successful impregnations. Winters[10] reported two successful impregnations with ram semen that had been kept in the laboratory for 6 days, three with semen kept 5 days, several with 3-day-old

[9] Table 5 appears on page 62.

[10] Proceedings of the conference on artificial insemination held in Chicago, November 27, 1936. Mimeographed report issued by L. M. Winters, secretary, University of Minnesota, St. Paul, Minn.

samples, while many were obtained with 1- and 2-day samples. Gunn (*12*) reported as great a percentage of impregnations in ewes with ejacula stored for 28 hours at 6° C. as with comparatively fresh ejacula used 4 to 6 hours after collection. Edwards, Walton, and Siebenga (*11*) report that successful impregnations of cows have been made in England with bull semen collected in the Netherlands; and investigators in the Bureau of Dairy Industry have shipped bull semen from Beltsville, Md., to Piran in the Province of Buenos Aires, Argentina, and according to a report from the Department of Agriculture of the Argentine Government to the Bureau of Dairy Industry at least one successful impregnation was obtained.[11] In this experiment 7 days lapsed between the time of collection of the semen and insemination.

If successful use is to be made of artificial insemination for such purposes, very careful coordination of effort is necessary between the persons shipping the semen and those doing the insemination. The shipments must be planned so that a minimum of time will elapse between collection and insemination, and it is necessary that the females to be inseminated be in heat and at the proper stage of heat, or estrus, that is shortly before ovulation, if a large percentage of conceptions is to be obtained. This requires a careful checking on the estrual cycles of any females to be inseminated in order that plans may be made to have the semen on hand at the proper time during subsequent heat periods of any females to be inseminated. In addition, it will be necessary to perfect apparatus for holding the temperature of the semen within the rather close limits of 3° to 8° C. for the interval of time required for shipment.

Because of these difficulties it is probable that such use of artificial insemination will be confined to the services of an outstanding sire that could not be obtained otherwise. With the widespread development of air transportation the shipment of semen may be of occasional use in obtaining the services of sires when the transportation of either the sires or dams would be too costly or otherwise not feasible. Its most effective use would seem to be at large experiment stations, on ranches, or in organized groups to spread the services of a sire over a large number of females.

PROBLEMS OF INSEMINATION

The percentage of successful impregnations to be obtained by use of artificial insemination is largely determined by the care with which the procedures are carried out. In the first place it is necessary to be sure that the semen contains large numbers of normal viable spermatozoa and that the females to be inseminated are normal, free from diseases of the reproductive stystem, and in good breeding condition. However, several other details must be observed. Among the important factors to be considered are: (1) Timing the inseminations so that they coincide closely with ovulation, (2) making more than one insemination in case of long heat periods, and (3) introducing an adequate quantity of semen into the proper region of the genital tract of the female. In addition, the handling of the female following insemination is sometimes an important factor in obtaining satisfactory results.

[11] Communication from the Bureau of Dairy Industry, U. S. Department of Agriculture.

Relation to Estrus and Ovulation

Insemination should be timed to precede ovulation by a few hours or to coincide with it. The life of spermatozoa in the reproductive tract of the female is relatively short. Although this interval is obviously subject to large variations, both within and between species, it is probable that the time seldom exceeds 40 hours and in most cases is much less, according to Phillips (*49*). In addition, the longer the interval after ovulation, the less are the chances of an egg being fertilized, because, as explained by the American physiologist Pincus (*51*), as it passes through the Fallopian tube it acquires a covering of albumen which interferes with fertilization.

The duration of heat in most species is relatively short but subject to considerable variation within the species. Ovulation occurs relatively late in estrus (table 2), p. 29 and inseminations should be timed to coincide closely with this interval. In practice the exact determination of time of onset of estrus is often impossible, but the breeder will be reasonably well assured of success if inseminations are made some time during the last half of estrus. The relationship between fertility and time of insemination (in relation to ovulation) in the rabbit is shown in figure 5. Ovulation occurs approximately 10 hours after coitus in the case of the rabbit.

In the case of females that have excessively long estrus periods, two or more inseminations or services may be advisable. Hammond (*14*) of England, states that it is good practice to serve a mare a second time if she is in heat 3 days after the first service, and Shchekin (*58*) of the Soviet Union recommends breeding on the second and fifth days, or if the stallion is not being used to excess, on the fourth, sixth, and eighth days after the onset of heat. Zivotkov (*75*, *76*) obtained 80.5 percent of pregnancies in mares bred between 4 and 48 hours before ovulation, as determined by palpation of the maturing follicle. He reports that mares begin to go out of heat from 6 to 12 hours after ovulation and that they will not accept the stallion after 24 to 48 hours. Breeding after ovulation results in few pregnancies. He recommends that the state of the ripening follicle be determined by means of palpation before insemination or service. To do this successfully a knowledge of the reproductive organs and skill in manipulation are required.

The investigations of Andrews and McKenzie (*3*) emphasize the value of palpating mare ovaries. Some mares do not express a desire to mate, yet the ovarian cyclic changes continue, and artificially inseminating these mares resulted in pregnancy. Other mares showed signs of estrus for 1 or more days, then went out for a day or so and came in estrus again. Ovulation occurred during the second phase of the "split" estrus. Still other mares showed estrus but failed to ovulate till after they had gone out of heat. Artificial insemination subsequent to the heat period resulted in impregnation.

Siebenga (*59*) reports that cows may be bred after estrus passes provided the cervix is moist with mucus. If there is no mucus on the cervix, insemination is useless.

According to Milovanov (*39*), some Russian sheep stations experienced a large increase in lambing percentages when the ewes were inseminated several times, and Neiman (*45*) reported similar results in Karakul sheep. Also, according to Neiman, two inseminations of

cows at intervals of 12 to 24 hours during one heat period resulted in 92.5 percent of pregnancies, as compared with 60 to 65 percent with a single insemination. Kirillov (*21*) states that the practice of inseminating cows once during the heat period is unsatisfactory and recommends that they be inseminated twice, first at the beginning and

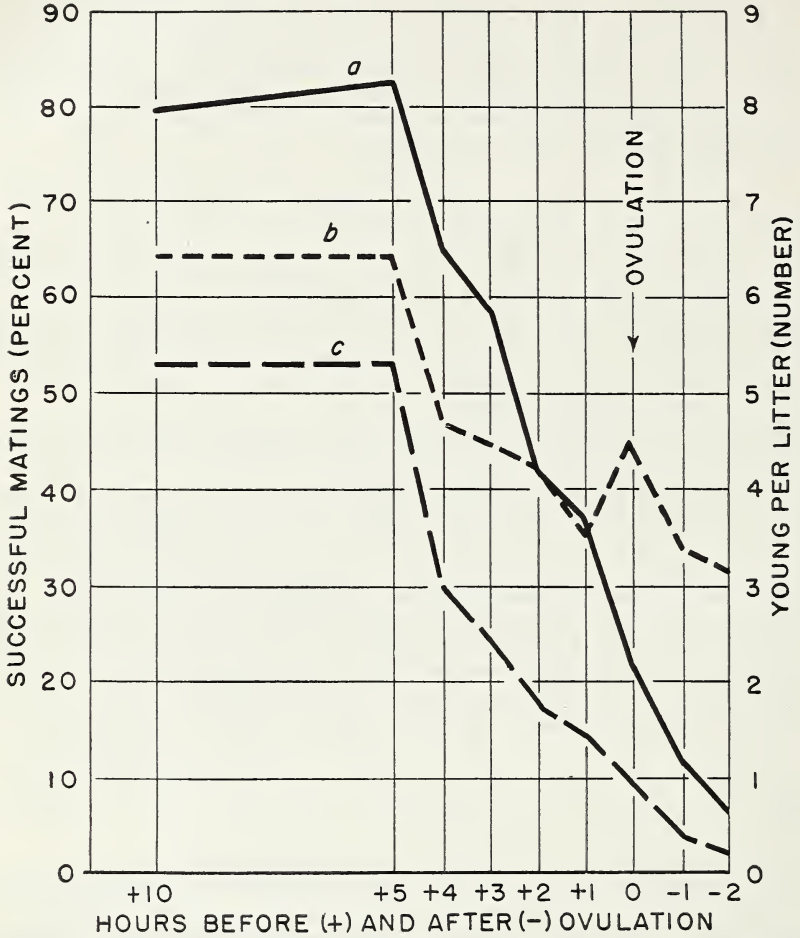

FIGURE 5.—Success of matings in relation to time of ovulation, for the rabbit. *a*, Percentage of matings that were successful; *b*, average litter size; *c*, number of young per mating. (In the rabbit ovulation occurs approximately 10 hours after coitus.) (Adapted from Hammond, courtesy of the Journal of Experimental Biology.)

again 18 to 24 hours later. When spermatozoa are of inferior quality such repeated inseminations are apt to be most effective.

Place of Injection and Quantity of Semen

The best results with artificial insemination are obtained by introducing the semen well into the cervix (fig. 6) or, with some species, directly into the uterus. Although there are few experimental data

on this question, such results as are available indicate that introduction of semen into the cervix is superior to introduction into the vagina. Walton (*65*) points out that in experiments with sheep where semen of high quality was introduced directly into the cervix the percentage of conceptions was 66 as contrasted with 33 percent for the same quantity introduced directly into the vagina. Miller [12] reported but little success following artificial inseminations when the semen was placed in the cow's vagina, although Kozlova (*24*) observed little difference between two groups of cows inseminated in these two

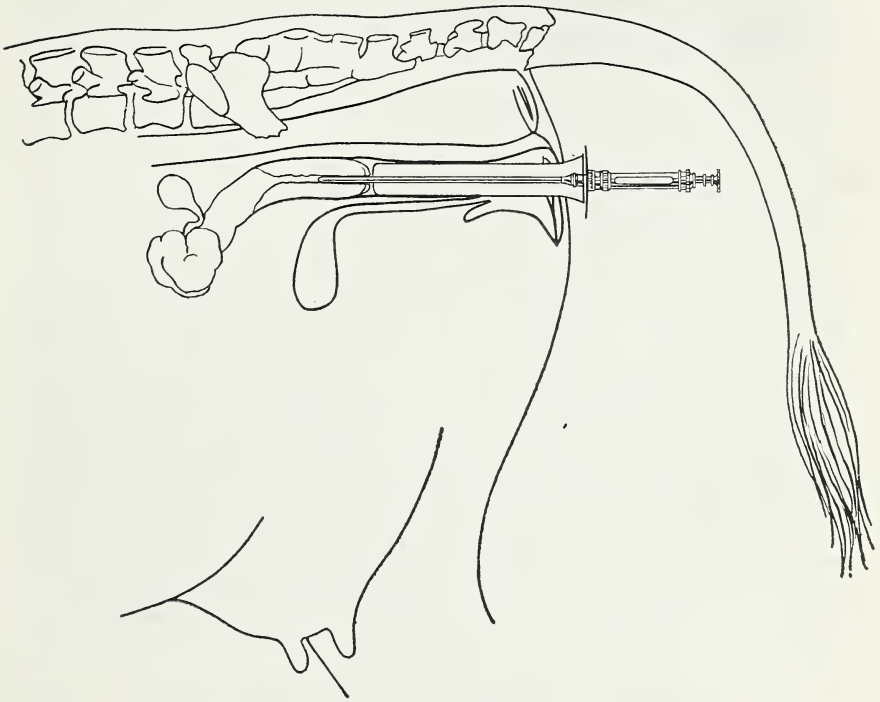

FIGURE 6.—Median section of the cow showing the reproductive organs in position with the inseminating syringe and tubular speculum in place. Nozzle of the syringe is shown inserted 1 to 2 cm. into the cervix.

ways. According to Walton (*65*), the semen should be introduced a distance of 1 to 2 cms. into the cervix of the cow and about 0.5 to 1.0 cm. into that of the ewe.

From inseminations made into different regions of the reproductive tract in normal cows Siebenga (*59*) reports the following percentages of pregnancies: Cervical insemination with undiluted semen, 89.6 percent from 199 inseminations; cervical insemination with diluted semen, 89.6 percent in 55 cases; cervical insemination where part of semen flowed back into vagina, 88 percent in 33 cases; uterine insemination with undiluted semen, 40 percent from 10 inseminations; and with vaginal insemination, 11.1 percent in 9 cases.

[12] Miller, FRED W. THE TECHNIC OF ARTIFICIAL INSEMINATION WITH DAIRY CATTLE. U. S. Bur. Dairy Indus. B. D. I. M. 738, 5 pp. [Mimeographed.]

In the sow it is probable that best results are obtained from introducing the semen directly into the uterus, where it is deposited during copulation, according to Rodin and Lipatov (*54*) and McKenzie, Miller, and Bauguess (*32*). Although conclusive experimental data are not available on this point for most species, it is probable that introduction of the semen into the cervix is superior to placing it in the vagina.

The quantity of semen to be used in artificial insemination is somewhat of a moot question. The quantity needed for successful fertilization is dependent upon such factors as the quality of the semen, including the number of viable sperm, the state of the reproductive tract of the female, the region into which the semen is introduced, and the stage of estrus. Neiman (*44*), of the Soviet Union, reports that quantities of semen in excess of 2 cc. did not increase the percentage of fertility in cattle, while quantities of 0.5 to 2 cc. were found to be most effective if the semen was introduced into the cervix. These results are in essential agreement with those of Kozlova (*24*) and Kufarev (*26*). In sheep the effective quantity was found to be from 0.1 to 0.2 cc., although inseminations may be obtained with quantities of 0.05 cc. Zivotkov (*75*) states that in the mare the quantity of semen should vary according to the age and size of the mare. He found that in young and small mares 10 cc. of semen is sufficient whereas for older and larger mares 20 to 25 cc. is best. He reports that the results were more favorable with such quantities than with volumes of 3 to 5 cc. In the sow much larger quantities are required for successful impregnations due to the exceedingly long and convoluted horns of the uterus, which sometimes reach a total length of about 2 meters or 78 inches. Rodin and Lipatov (*54*) recommend 100 to 200 cc., depending on the size of the sow. McKenzie, Miller, and Bauguess (*32*) found that 50 cc. of boar semen made free of gelatinous particles was sufficient. In rabbits Walton (*65*) reports that satisfactory results are obtained with 0.5 cc. of semen which has been diluted as much as 32 times. In the chicken Burrows and Quinn (*9*) state that insemination with 0.1 cc. of semen once a week should result in fertility in 80 to 95 percent of the eggs.

Conditioning the Male for Breeding

An important consideration in all breeding programs, whether the program involves natural breeding or artificial insemination, is that the male be kept in the best possible breeding condition. This condition is described by the English investigators, Marshall and Hammond (*35*), as a "hard one produced by sufficient exercise to work off a surplus of fat, but favouring the retention of nitrogenous substances and vitamins." The management program should be such that breeding males and females should be improving in condition throughout the breeding season, for an improving condition is more favorable to the normal functioning of the reproductive system than a stationary or falling condition. Excessive fatness in breeding males should be avoided. Although excessively fat males may not be sterile, their fertility is lowered either by the production of fewer spermatozoa or of spermatozoa of lower quality.

The ration of the male should always be properly balanced, containing ample supplies of proteins, minerals, carbohydrates, and vita-

mins. The particular constituents entering into a ration will vary from region to region and to some extent with price changes in various feedstuffs.[13] Where possible, the male should be allowed access to or be given green feed for several weeks before and during the breeding season, and he should have access to an ample supply of good water at all times.

Ample exercise should be provided for all breeding males since proper exercise is essential to the best functioning of the body and for the maintenance of appetite and a generally thrifty condition. Care should be taken to avoid overheating, especially in warm weather. Males receiving an adequate amount of exercise will produce, on an average, larger ejaculates containing more sperm and a higher quality of sperm. The males will also be more active and will deliver more services in a given interval of time.

Where summer temperatures are frequently above 80° to 85° F. it is good practice to allow the male access to cool quarters, cool water for drinking, and ample shade. It is important to avoid excessively high temperatures throughout the summer. Unpublished work done at the Missouri Agricultural Experiment Station indicates that Shropshire and Hampshire rams protected from temperatures above 80° produced more semen and a larger number of normal sperm and were prepared to enter the breeding season earlier than similar rams subjected to the usual temperatures of 80° to 100°.

When males are to be used extensively for artificial insemination, it is very important that they should be docile, well broken to lead, and free from bad habits. Most males will serve best in familiar surroundings, and if possible the male's own or at least the same paddock should be used for breeding purposes. Following a regular daily routine and handling the male in the same manner each time are a very essential phase of good management. The male should be trained to mount the female without undue delay, although too rapid mounting should be discouraged for there is evidence according to the Russian investigators Kirillov and Morosov, (*23*) that a reasonable amount of time expended by the male in maneuvering previous to service results in a larger quantity of semen and better quality of spermatozoa. Also, McKenzie and Berliner (*31*) have shown that the number of sperm was less in the first ejaculate of rams that showed a strong sexual impulse and mounted the ewe very quickly than it was in subsequent ejaculates. Obviously much individual variation exists in this respect, and for best results an operator should make regular semen examinations of each sire that is to be used extensively. Examinations made weekly or twice a month are not too frequent.

Males can easily be trained to mount females out of heat and even to mount dummies (fig. 7). This may be accomplished by first letting the male become accustomed to serve a female in heat in the same stall or in the same breeding crate. A female out of heat or even a dummy may then be substituted. In the boar, according to Rodolfo (*56*), sexual attraction plays very little part in the mating behavior as this animal is easily stimulated to attempt mating by the presence of a dummy sow.

[13] The reader is referred to publications of the U. S. Department of Agriculture and State experiment stations, or to Morrison (*42*) for information pertaining to the feeding of livestock.

Frequency of Service and Reproductive Capacity

Much work has been done in an attempt to evaluate the influence of frequency of service on reproductive efficiency and the quality of spermatozoa for both natural and artificial insemination. Great variation in the rate of exhaustion of spermatozoa in successive ejaculates exists between the different species and to a lesser extent even in the same species. In like manner, much variation exists in the rate of replenishment of the sperm supply following periods of rest after sexual exploitation according to McKenzie and Berliner (*31*). For those species that produce a large volume of ejaculate at each mating, such as the horse, boar, and dog, the sperm number per ejaculate decreases rapidly, as well as the viability of the sperm, while for other species like the bull and ram which ejaculate relatively small quantities of semen at one time, a great many matings may be made in a relatively

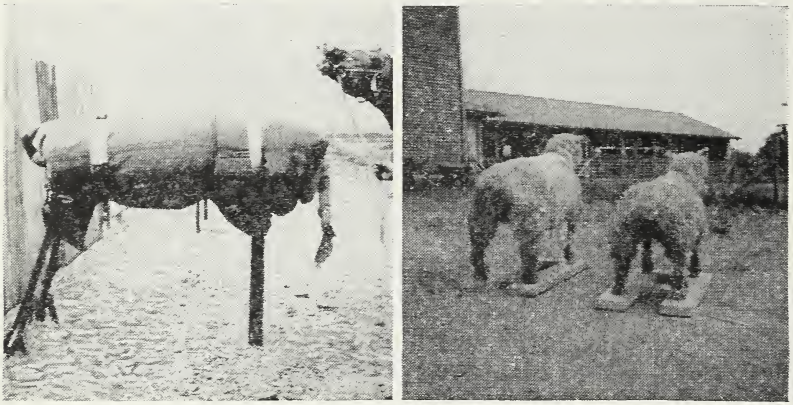

Figure 7.—Dummies used for the collection of semen at the animal husbandry experiment stations at Miles City, Mont., and Beltsville, Md. Note that two sizes of sheep dummies are used to accommodate different sizes of rams. The framework of each dummy is of metal which is either bolted to the platform or firmly implanted in the ground as shown at left. Note that ample space is provided under sheep dummies for insertion of the artificial vagina which is held in place by rubber bands or by the operator.

short interval without greatly decreasing the number of sperms per ejaculate or their viability.

For many mature stallions it would appear that two to five matings on some days may be made without lowering the average level of fertility. Good management, however, would seem to call for lighter use following days with heavy breeding schedules. According to Polowzow (*52*), a rest period of 24 hours is sufficient to restore the number of sperm to normal and to eliminate the high proportion of nonviable sperm that were evident after frequent matings.

In the boar, whose volume of semen per ejaculate is the largest among domesticated animals, there should not be more than one mating a day, according to Rodolfo (*55*), and even then there should be an interval for rest after every 2 days. This is particularly true for the yearling boar, which probably should not be used oftener than once every other day if he is to be used for as long as 2 weeks; otherwise the number of sperm decreases markedly, and immature sperm

begin to predominate, as explained by McKenzie, Miller, and Bauguess (*32*).

Much experimental evidence indicates that in bulls and rams many successive matings may be made in 1 day without depleting the sperm supply, as shown by McKenzie and Berliner (*31*). For bulls Kirillov (*22*) suggests that for prolonged use two matings a day is the optimum number. His observations indicate that three services daily resulted in decreased sexual potency. In rams it would appear that more than three matings a day can be made without serious depletion of spermatozoa. While the number of spermatozoa decreases in each ejaculate with successive matings, the sperm supply never seems to be permanently exhausted through successive matings, and a short rest period seems to suffice for a fresh supply of sperm to be produced. Much variation is observed between individual rams in these respects, and there is also seasonal variability in the rate of sperm production.

Libido, or sexual desire, is not a reliable index of sperm-producing capacity of males. For instance, in sterile males or in double cryptorchid males in which the spermatogenic function is absent, the mating desire may be very high, and good rams may continue to copulate when the supply of spermatozoa has been temporarily exhausted by frequent matings. However, in those species which have a more or less well-defined breeding season it has been shown by McKenzie and Berliner (*31*) that more copulations occur during periods of high fertility and that there is a higher rate of sperm production during such periods. In dogs, Alifanow (*1*) observed a correlation between sex interest and sperm production.

THE PRACTICE OF ARTIFICIAL INSEMINATION

General Considerations

In the practice of artificial insemination, regardless of the species, there are certain common procedures that must be followed as well as certain precautions that must be taken to insure the greatest success. Before undertaking insemination it is necessary that the proper apparatus be on hand and that it be clean, free from harmful chemical substances or bacteria, and completely dry. The instruments should be placed on a clean towel or blotting paper on a solid but movable table or stand in close proximity to the operator, and they should be kept covered with a clean towel to keep them free of dust. The hands of the operator should be thoroughly washed with soap and hot water, then rinsed and dried, preceding any collections or inseminations.

The paddock or quarters should be ample in size, level, and free from any objects that might frighten or injure breeding animals. With the larger animals smooth concrete floors should not be used because of the danger to the animals from slipping, especially when the floor becomes wet following urination. If possible the same paddock or breeding quarters should be used each time, since males work best in familiar surroundings and they soon learn to anticipate service when led into these quarters. The crates or other equipment should be arranged so as to be most convenient for the operator for both collection and insemination.

A very important consideration for success is to time inseminations so that they will be made shortly before ovulation because after ovula-

tion eggs soon lose their capacity for fertilization and the life of spermatozoa in the female reproductive tract is rather short. If a determination of the approximate time of the onset of estrus is not possible, two or more inseminations should be made during the cycle, especially for the mare, as in this species estrus occasionally lasts for 15 days or more. In general, ovulation occurs near the end of estrus although considerable variation in this respect exists between different animals as well as in the duration of estrus and the intensity of manifestation of heat. The optimum periods for insemination in the various species are shown in table 2.

Insemination should be made in clean, dust-free quarters in order to prevent exposure of the vaginal mucosa of the female to infectious agents that might be carried in dust particles. If inseminations are to be made in paddocks or barns, these may be rendered temporarily dust free by sprinkling the floor shortly before inseminations are made.

Three more or less distinct processes are involved in the practice of artificial insemination. These are: (1) Collection of the semen, (2) examination of the semen and preparation for insemination, and (3) insemination of the female. Since the procedures and equipment needed vary somewhat for the different species, they are discussed separately for each species. Only the easier and more successful means for the collection of semen are presented in the following discussion. For a discussion of other methods the reader is referred to the section entitled "The Collection of Semen" and to the papers listed in the appended literature citations.

Cleaning and Preparing the Apparatus

As spermatozoa are easily injured or killed by toxic agents of many sorts, it is necessary that all the apparatus be free from harmful chemical agents as well as free from harmful bacteria and molds. Accordingly, all apparatus must be thoroughly washed and disinfected before and after each use. Rigid adherence to this practice will insure the best viability of spermatozoa and is essential to maintenance of health in a herd or flock.

All new apparatus should be washed thoroughly with hot water and soap in order to remove all grease and other chemical substances that may be adhering to it. After being washed, it should be carefully rinsed in several changes of clean water, preferably distilled water, and then allowed to stand from 5 to 10 minutes in a 65-percent solution of ethyl alcohol, which is a disinfecting agent. It should then be allowed to dry in a clean, dust-free place, or, if required for immediate use, it may be rinsed in several changes of physiological saline solution (0.9 percent) to remove the alcohol. Dry heat and flaming are excellent means for sterilizing glassware where facilities are available, but precautions must be taken against too rapid heating or cooling, which may result in breakage. Denatured or wood alcohols are not suitable for disinfection purposes since they contain chemical substances that are injurious to spermatozoa.

Following each use, all apparatus should be cleaned by being scrubbed thoroughly with a brush in hot water and then rinsed in ethyl alcohol, as previously described. The use of soap subsequent to the cleaning of new apparatus is not to be recommended for traces of soap are injurious to spermatozoa and soap is somewhat harmful to rubber apparatus.

TABLE 2.—*Duration and frequency of estrus and time of ovulation in farm animals in normal condition*

Animal	Duration of heat				Length of estrual cycle [1]				Approximate time of ovulation in relation to heat	Optimum time to breed in heat period	Remarks
	Approximate range		Most common duration		Range		Most common duration				
	Days	*Hours*	*Days*	*Hours*	*Days*	*Hours*	*Days*	*Hours*			
Mare	1–37		3–7		10–37		18–24		2 days before end of heat until 1 day after heat	If feasible, once daily after first day of heat in light mares, after second day in draft; if bred only once, on the third day. When the mare is in heat 3 days after breeding, breed a second time. If the ovary is palpated, breed when there is a large, slightly relaxed ovarian follicle, 2–5 cm. in diameter.	Foal heat in mares usually lasts 5 days, but may range from 1–10 days. Mares usually come into heat 5–10 days after foaling.
Cow		12–18		16	19–23		20		20–40 hours after onset of heat	Preferably twice, once shortly after onset of heat and again 12–20 hours after onset; if but once, 12–20 hours after onset.	
Ewe		20–42		30	14–19		16–17		About 1 hour before end of heat	During the last half of heat, or, if feasible, at 12-hour intervals as long as in heat.	There is evidence that the duration of heat is longer in some breeds, as the Lincoln and Corriedale, the mean duration being about 40 hours.
Doe (goat)		20–80		39	12–27		19		Usually on the second day of heat	During last half of heat period.	
Sow, mature	2–4		3		19–23		20–22		Early on the second day of heat	Late on first day or preferably on second day of heat.	Sows usually come into heat 3–4 days after weaning pigs; earlier if the litter is small.
Gilt		40–48	2								
Bitch	4–13		9		126–240		180		24–48 hours after onset of heat (first acceptance of coitus)	On eleventh to thirteenth day after beginning to bleed.	Much individual variability exists in estrual cycle. Each bitch usually remains quite constant to 1 particular period. Length of cycle is influenced to some extent by such factors as breed and age.
Doe (rabbit)									8–10 hours after coitus	Breeding again 2 to 5 hours after first coitus increases litter size. Breeding later than 5 hours after ovulation reduces litter size.	No regular estrual cycle exists, but there is evidence that there are certain periods of greater receptivity. If nutritive conditions are favorable, and the does are not molting and are in proper breeding condition, they may be mated at any time if restrained.

[1] The length of the estrual cycle is the interval from the beginning of 1 heat period to the beginning of the next.

General Equipment Needed

The equipment and supplies needed for collection and insemination vary considerably for the different species, but certain items are needed regardless of the species used. Such items are listed here, but special items needed for the different species are listed under the respective species. General equipment and supplies that are convenient to have on hand are:

1. A supply of 65-percent ethyl alcohol (not denatured).
2. Several rolls of absorbent cotton in sterile packages.
3. A good lubricant (see section on Collection of semen by approved methods for formula).
4. Rubber gloves.
5. A flashlight or small head lamp with battery.
6. A supply of clean, freshly laundered cheesecloth. New, unwashed material should be avoided.
7. A bar of good-quality hand soap.
8. A supply of test tubes or small bottles (the size depending on the species) in which the semen may be placed after collection. It is desirable to use tubes or bottles of Pyrex or otherwise of a quality not likely to break when heated or cooled.
9. Rubber stoppers for the bottles and test tubes.
10. A test-tube rack or stand for the bottles. The rack or stand should have a broad base so that it will not easily upset.
11. A pipette case for holding pipettes, glass tubes, or ebonite nozzles. A layer of cotton should be placed in each end of the case to prevent breakage.
12. An insulated container in which to put the semen after collection or in which to transport it short distances (vacuum bottle, pint or quart size).
13. A glass (wax) pencil or gummed labels for labeling the tubes or vials.
14. An enameled pan about 12 by 15 by 2 inches in which equipment may be placed after use.
15. A supply of distilled water and salt for preparation of physiological saline solution.
16. Two graduated beakers for preparing solutions, 100-cc. and 500-cc. sizes.
17. Several glass beakers, preferably of Pyrex, 100, 250, and 500 cc.
18. A box of clean glass slides and cover slips.
19. One hollow-ground slide for making motility observations.
20. A slide box for storing slides after preparation.
21. A thermometer for measuring the temperature of water for the artificial vagina, etc. (0° to 110° C.).
22. A dozen medicinal droppers.

It is well to have a surgical and first-aid kit for use in case of injury to man or animals. All the materials and apparatus should be kept in a clean, dust-free place, and it should be made ready for use before collections or inseminations are begun.

If complete examination of the semen is to be made, a microscope with magnifications up to 300 or 400 diameters, a hemocytometer, and pipette or burette, and a supply of stains (p. 16) are needed in addition to the items listed. Such examinations are best made in the laboratory, preferably by an experienced technician as considerable experience and skill are required for an adequate examination.

Artificial Insemination of Horses and Asses

The stallion is handled in the same way as in normal service, preferably by a groom who is familiar with the procedure to be followed. The mare also should be handled by a groom, and there should be assurance that she is in heat. If the mare is inclined to be nervous or unruly, hobbles, and in extreme cases a twitch, should be used to restrain the mare and avoid injury to the operator and the stallion.

but if the mare is quiet and fully in heat there is little likelihood of trouble. Always handle breeding stock gently. Avoid startling any animal. Place the mare in such a position that she can see the stallion or jack approaching. It is good practice to wash the penis of the stallion at regular intervals with soap and water to remove any dried secretions ("scales") and then rinse well with clear water. The tail of the mare should be bandaged and tied. If the collection is to be made from the mare, the area around the tail region should be washed well with soap and warm water or a mild solution of a nonirritating disinfectant in warm water,[14] then rinsed with clean water. The lips of the vulva should be spread and swabbed with cotton. If it seems desirable to flush out or douche the vagina before breeding, a solution made up as follows may be used: 1 quart of water; 2½ tablespoonfuls of salt; and 1 tablespoonful of baking soda. The water should first be boiled to sterilize it and the salt and soda then added. Before being used the solution should be allowed to cool to body temperature. Do not breed or inseminate a mare until 2 hours after a douching.

The apparatus and supplies needed will vary with the method of collection. The following list gives the essential items:

Hobbles, to restrain the mare.

A cotton tail-bandage, 5 feet long and 2 inches wide.

Two buckets of approximately 10-quart capacity and preferably of enameled ware.

A speculum. This item is not absolutely necessary but is desirable for its use makes it possible for the operator to observe the interior of the vagina and the cervix.

Equipment for the collection of semen:

An artificial vagina (Missouri-U. S. D. A. model); or one 6- or 8-ounce bottle with a two-hole rubber stopper, fitted with glass tubes and rubber tubing (fig. 8); or a breeder's bag. If a large number of collections is to be made it is desirable to have all types of collecting equipment on hand.

Equipment for insemination:

Gelatin capsules (2 and 4 drams; ½ and 1 ounce, i. e., Nos. 11 and 10) which should be kept in a clean, tightly-stoppered glass jar; or a 20-cc. glass syringe with glass plunger fitted with a 20-inch ebonite or glass nozzle which has well-rounded and smooth ends. Metal syringes should be avoided.

Collection of Semen From the Vagina

For the horse this is one of the more satisfactory means for collection of semen, but due precaution must be exercised to see that the mare used for collection is free from disease. Nor should a mare in foal heat be used for collection since the danger from infection is too great at this time both for the mare used for collection and for the mares to be inseminated with semen so collected. A healthy mare that is in heat is hobbled and prepared as indicated above. After the male dismounts, the operator puts on rubber gloves, washes thoroughly, lubricates [15] the gloves and the vulvar lips of the mare, and with the long rubber tube of the semen collector (fig. 8) between the first and second fingers, carries the tube into the vagina and draws off the semen by aspiration, that is, by sucking on the short rubber tube. If the semen is within the cervix, the tube is guided into the cervix, and the semen is aspirated from there. If the weather is cold,

[14] 20 cc. of a high-grade coal-tar creosote disinfectant per gallon of warm water is very satisfactory.

[15] See Collection of Semen by Approved Methods for formula of lubricant.

protect the semen from sudden changes of temperature by keeping the semen bottle wrapped in a warm towel.

Another but less satisfactory means of collection following service is by use of the speculum and a syringe. After service a speculum may be carefully inserted into the vagina, opened, and then a nozzle which is attached to the syringe is carefully inserted into the vagina and the semen withdrawn into the syringe. The semen is deposited chiefly on the floor of the vagina just posterior to the cervix. After the semen is withdrawn it should be handled as described in the preceding paragraph.

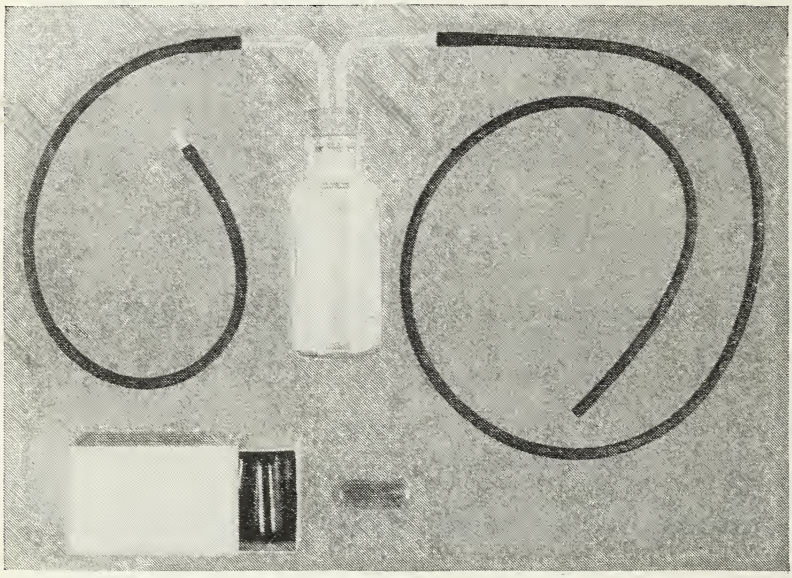

FIGURE 8.—Apparatus for aspirating semen from the mare and gelatin capsules (½ ounce) for insemination. A 6-ounce bottle with heavy-walled gum-rubber tubing of 3/16-inch inner diameter is used. The gelatin capsules should be kept in a small, tight container such as a paper box or screw-top bottle. (Courtesy Missouri Agricultural Experiment Station.)

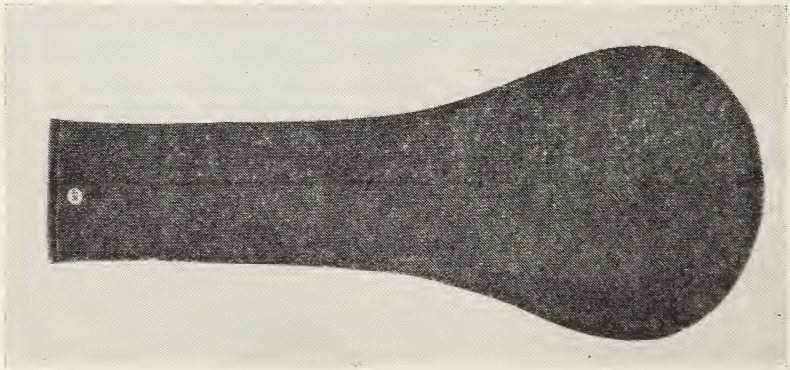

FIGURE 9.—Breeder's bag. Size for draft horses is 15 by 3 inches (flat diameter of neck); for light horses, 13½ by 2¼ inches. (Courtesy Missouri Agricultural Experiment Station.)

COLLECTION BY MEANS OF THE BREEDER'S BAG

If the stallion or jack will permit the use of the breeder's bag (fig. 9), it offers a very satisfactory and relatively simple means of collecting large volumes of semen free from female secretions. Place a breeder's bag of good quality and proper size on the penis while the stallion is getting the erection. Lubricate the outside bulb end of the bag and the external genitals of the mare. Permit service and after he has dismounted take the bag from the penis, pour the semen into a bottle, and cork the bottle. Protect the material from direct sunlight and sudden changes in temperature.

COLLECTION BY MEANS OF THE ARTIFICIAL VAGINA

The collection of semen by means of the artificial vagina (figs. 10 and 11) has certain advantages over the previously described methods,

FIGURE 10.—Artificial vagina for the horse, Missouri-U. S. D. A. model. A device for fractionating the semen is attached to the lower (small) end of the apparatus.

from the standpoint of quantity and quality of the semen obtained and in the fact that danger of infection is greatly minimized. The disadvantages are that the artificial vagina for the horse is rather expensive, especially if it is to be used only for occasional inseminations, and the temperature and pressure must be carefully regulated if the stallion is to be induced to use this device.

The Missouri-U. S. D. A. model of the artificial vagina for the horse consists simply of a rubber tube about 18 inches long and 7

inches in flat diameter with a rubber ring placed in the open end, and the other end narrowed down to stretch over a bottle. In order to develop the proper pressure a second rubber tube of the same size is drawn over the first, the two tubes are then vulcanized together at each end, and an ordinary tire valve is placed in the outer tube so that air may be pumped between the two tubes. A leather casing surrounds the rubber tubing, giving it rigidity, and a handle grip is attached to the casing. An essential feature of this model is the 3-inch rubber band placed around the inner tube near the open end. This simulates the sphincter muscles of the mare and aids materially in making collections from stallions. The apparatus is warmed by passing hot water through it just prior to use and flushing with either physiological saline solution (0.9 percent) or a good semen diluter. If the valve is large enough, pour a quart of hot water through it to warm the apparatus, and thus make flushing with saline unnec-

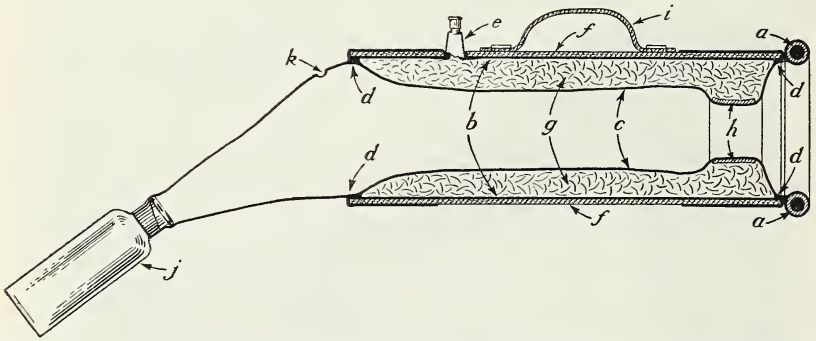

FIGURE 11.—Longitudinal section of the Missouri-U. S. D. A. model artificial vagina for the horse. Length 18 inches, 7-inch rubber tubing, flat diameter; *a*, Entrance ring made by enclosing section of ½-inch garden hose; *b*, outer tube; *c*, inner tube; *d*, points at which the outer tube is vulcanized to the inner tube; *e*, air valve; *f*, leather casing to give support and rigidity; *g*, air space between inner and outer tube which allows for the adjustment of pressure; *h*, the "sphincter" rubber band, made of 3-inch flat-diameter tubing 1½ to 2 inches broad; *i*, handle grip attached to leather casing; *j*, 8-ounce collecting bottle; *k*, air vent to prevent ballooning.

essary. Before attaching the collecting device, previous to use, as much of the flushing agent as possible should be shaken out.

COLLECTION OF SEMEN AT THE TIME OF WITHDRAWAL

This is the simplest method of collection but the portion of the semen collected by this method is relatively low in sperm count. In this method the male is permitted to serve the female in the usual manner and as he dismounts the remaining semen is caught in an enameled dipper. It should then be poured into a test tube or glass vial and covered. The receptacle, both dipper and container, should be warmed to body temperature, and in cold weather care should be taken to avoid sudden changes in temperature by wrapping the container in a towel.

EXAMINATION OF THE SEMEN AND PREPARATION FOR INSEMINATION

Stallion semen collected in the spring usually contains three fractions ejaculated in sequence. The first fraction (5 to 10 cc.) is watery, often grayish in color and contains few or no sperm. The second fraction (25 to 75 cc.) is thin or watery in consistency and high in sperm concentration, 50,000 to 400,000 or more per cubic millimeter. The third fraction is viscous and, if present at all, may be the most voluminous constituting 44 to 71 percent of the total volume of the ejaculate. Jacks have not been observed to produce much of the viscous fraction (except in old age) and ordinarily stallions do not produce it in the fall. This viscous portion comes primarily from the seminal vesicles and the Cowper's glands and liquefies soon on standing, and it is well to include it along with the high sperm containing fraction in the material placed in the mare at the time of insemination.

The semen should be kept cool if it is to be retained for 2 to 6 hours and should not be exposed to direct sunlight. The optimum temperature for storage is about 10° C.

If a stallion is to be used extensively, careful examination of the semen should be made at frequent intervals for volume, color, sperm concentration, motility, and abnormalities. The details of such examinations have been described in the section, Examination of the Semen.

INSEMINATION

The mare or mares to be inseminated should be tried with the stallion to be sure that they are in heat. As a precaution against kicking, the mare should be hobbled, and if further restraint seems necessary, a twitch may be used or the foreleg held up. The mare should be stood facing a dark or shaded wall with her tail to the light. The vulva should be wiped clean, then dried, and the inner lips swabbed.

A simple and convenient method of insemination is to use a gelatin capsule, either a ½-ounce or 1-ounce capsule. From 10 to 30 cc. of semen (table 3) should be poured into the capsule and the capsule closed. It should then be carried into the vagina without delay by the hand and placed well forward in the cervix. For this operation the hand and arm of the operator should be clean and well-lubricated. Any rough or sharp edges of the fingernails should be removed. If a rubber glove and obstetrical sleeve are used in capsuling mares, infection can be kept to a minimum.

When an inseminator (syringe) is used for insemination a speculum should first be coated with a lubricant and introduced into the vagina by gentle rotation and pressure exerted in an upward direction until it passes over the brim of the pelvis, care being taken to avoid

injury to the urethral meatus during insertion. The cervix should be clearly visible. An assistant should rinse the syringe with a little of the seminal fluid and then fill it completely with this fluid. All bubbles in the syringe and nozzle should be eliminated; this may be done by holding the syringe perpendicularly and forcing the bubbles out by a gentle upward pressure on the plunger. The nozzle of the syringe should then be carefully inserted through the cervix and the semen slowly injected into the uterus. From 10 to 30 cc. of semen should be injected, the amount depending on the number of mares to be inseminated and the quantity of semen available. It should be emphasized that use of the speculum always exposes the vagina to infection with bacteria or other microorganisms carried in the air.

TABLE 3. *Some quantitative characteristics of semen from various animals* [1]

Animal	Volume per ejaculate		Sperm concentration (per cubic millimeter)		Hydrogen-ion concentration	Volume of diluted or undiluted semen recommended per artificial insemination [2]
	Approximate range	Most common volume	Range	Most common		
	Cubic centimeters	*Cubic centimeters*	*Number*	*Number*	*pH*	*Cubic centimeters*
Stallion	40–320	75–150	30,000–800,000	60,000	7.0–7.8	10.0–30.0
Bull	0.5–14.0	3.0–4.0	300,000–2,000,000	800,000	6.5–7.5	.5–1.5
Ram	.5–2.0	.8	500,000–6,000,000	1,000,000	6.2–6.8	.1–0.2
Boar	125–500	200.0	25,000–1,000,000	100,000	6.8–7.2	50.0–100.0
Dog	2–19	7.0	1,000,000–9,000,000			
Fox	.1–4.5	1.5			6.2–6.4	
Buck (rabbit)	.4–6.5	0.7	100,000–2,000,000	700,000	6.8–7.5	.25–1.0
Turkey tom	.1–0.7	.3				.05
Cock	.1–1.5	.6	50,000–6,000,000		7.3–7.8	[3] .1

[1] No data are available for the goat.
[2] The volume here recommended is for cervical insemination. In birds the semen is introduced directly into the oviduct.
[3] Inseminations should be made at least at weekly intervals, preferably every 3 to 4 days.

Insemination may be made also by the operator introducing his arm into the vagina and inserting the tip of a rubber catheter through the cervix. The semen is injected by means of a syringe which is attached to the outer end of the catheter. The assistant should fill and operate it in the manner described in the previous paragraph and care should be exercised to expel all air bubbles before the catheter is introduced into the vagina. The operator gently works the tip of the catheter through the cervix. In mares fully in heat this is rather easily done, but if difficulty is encountered due to rigidity of the cervix this usually can be overcome by gently massaging the cervix for a few minutes. Before the arm is introduced into the vagina it should be washed and lubricated and any rough or sharp edges on the fingernails should be removed. Wearing a rubber glove reduces greatly the chances for infection. This method may also be used effectively for collecting the semen from the vagina of a mare and introducing it into the cervix of the same mare following natural mating. The practice is sometimes valuable in cases of mares that are difficult to settle. This method of inseminating is unusually hazardous because of the difficulty in keeping this type of equipment clean.

If possible, mares should be inseminated near the end of the heat period since ovulation occurs about 24 hours before heat ends. The heat period normally is long, ranging from 3 to 7 days and varying from 1 to 15 days or more. It is often difficult, therefore, to tell just when heat begins, and it is sometimes desirable to inseminate twice during the period. Such practice is especially desirable in females which have extra-long heat periods and which are difficult to get settled. Breeding the third, fifth, and seventh days, if the mare is still in heat, is considered good practice as the chances for inseminating at the proper time are greatly increased.

Artificial Insemination of Cattle

The procedures to be followed in the collection of semen and for insemination in cattle are similar to those used for horses. Before any operations are undertaken all apparatus should be carefully cleaned, made ready for use, and placed on a table or stand that is convenient to the operator. The apparatus needed will depend somewhat on the method used for collection. The following is a complete list of equipment needed for collection and insemination regardless of the method used:

Two small glass funnels, preferably of Pyrex. (Only one is needed, but it is best to have an extra in case of breakage.)
A test-tube brush with medium-soft bristles.
A 2-cc. graduated glass syringe.
A heifer-size speculum.
An 18-inch ebonite nozzle equipped for attaching to the syringe. A glass tube with ⅛-inch inner diameter may be substituted for the nozzle. The ends should be well rounded in a flame and connected to the 2-cc. glass syringe by short pieces of rubber tubing (fig. 14).
An artificial vagina (Swedish or Russian type) and one spare inner tube. (This equipment is needed only in case this method of collection is to be used).

If the semen is to be collected by means of massage, the bull should be tied in a stall. However, if semen collection is to be made from the vagina of the cow or by use of the artificial vagina, the bull is handled as for normal mating. He should be led up to the cow with a staff or halter, this depending upon his disposition. The safety of the operator always should be assured. The paddock should be sufficiently large to allow plenty of room for turning, and the floor should be level and of such nature that there is little danger of slipping. If possible the same yard should be used for all services as a bull soon learns to anticipate service when led into a yard which is used for that purpose.

For vaginal collections, the cow may or may not be in heat, but in all cases she should be free from diseases of an infectious nature. In no case should a cow that has just calved be used for vaginal collections. She may be tied, held with a halter, or preferably placed in a breeding rack or chute (fig. 12). The region around the vulva should be washed well with warm water and soap, then rinsed with clear water, and the lips of the vulva spread and swabbed with clean cotton.

COLLECTION OF SEMEN BY MEANS OF MASSAGE

Semen may be collected directly from the bull by massage of the ampullae of the vasa deferentia, but if this method is to be used a knowledge of the bull's anatomy, or instruction in the technique, is

necessary. The details of the method, which was devised by Miller and Evans (*36*), are as follows: The bull is tied in place in a stall in such a manner that he cannot shift from side to side, and the sheath is carefully washed with a soft brush and warm water. This usually stimulates urination, which is desirable as it helps prevent contamination of the semen with urine. A rubber glove is placed on the hand and well lubricated. The gloved hand is then inserted in the bull's rectum a distance of 7 to 10 inches and the seminal vesicles (fig. 13, *A*) massaged by backward strokes and their contents, a turbid fluid contain-

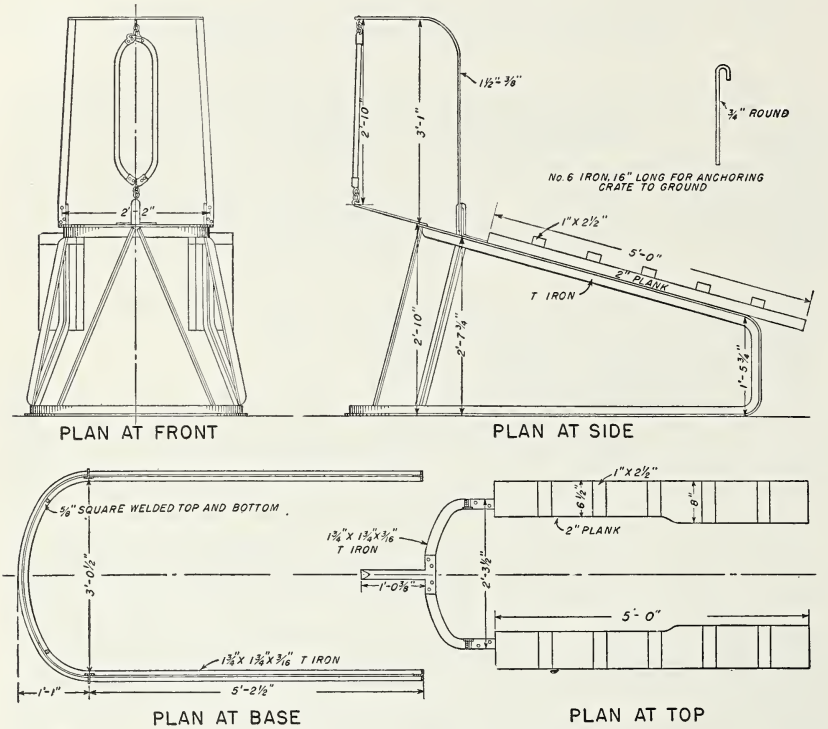

FIGURE 12.—Detailed plans of a good movable breeding crate for cattle. (From Edwards and Walton, courtesy Empire Journal of Experimental Agriculture.)

ing relatively few spermatozoa, thus expelled. After massage of the seminal vesicles the ampullae (widened ends) of the vasa deferentia (fig. 13, *B*) are massaged in a similar manner. The fluid from the latter ducts is very rich in spermatozoa. Miller and Evans reported 81 successful collections from 100 massages on 15 different bulls. In none of these cases was there apparent injury to the bull. The quantity of fluid collected from the seminal vesicles at one massage ranged from 0.5 to 21 cc. and that from the ampullae from 0.5 to 23 cc. The small quantities were thought to be due to the emptying of the ampullae shortly before they were massaged. Sometimes the ejaculate is retained in the sigmoid flexure of the penis. To avoid this, the operator should straighten this flexure with his other hand after massaging the ampullae.

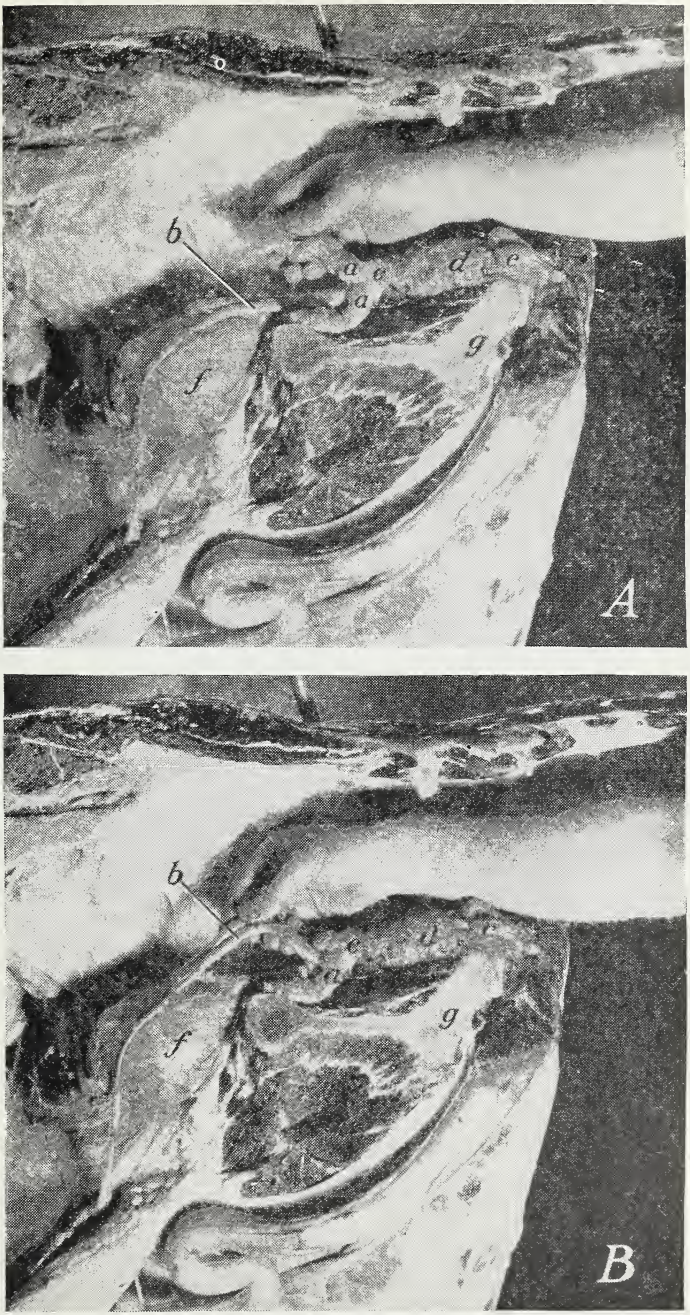

FIGURE 13.—Median section of the bull showing the reproductive organs in position and the method of manipulating them for the collection of semen by massage. *A*, Massaging the seminal vesicles; *B*, massaging the ampullae of the vasa deferentia. The organs are: *a*, Seminal vesicles; *b*, ampullae; *c*, body of prostate; *d*, pelvic urethra; *e*, bulbo-urethral (Cowper's) glands; *f*, urinary bladder; *g*, pubis. (After Miller and Evans.)

The semen is collected from the end of the penis or sheath by means of a funnel and test tube held by an assistant, and after collection it is handled in the same manner as semen collected by other methods.

COLLECTION FROM THE VAGINA

This is the simplest method for collection of semen. The semen is collected from the floor of the vagina, following copulation, by a long-handled vaginal spoon (fig. 2). Semen may be withdrawn from the vagina also by means of a syringe and nozzle (fig. 14). Although the semen may be withdrawn without using a speculum, its use facilitates more complete collection of the ejaculate, there is less chance of

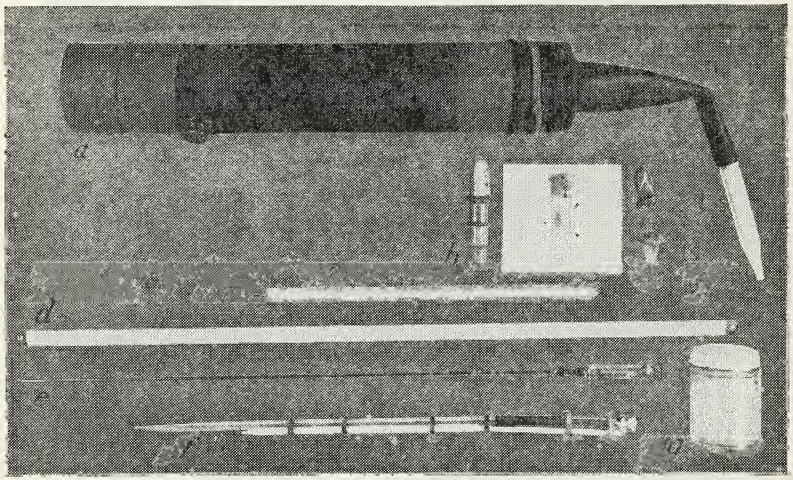

FIGURE 14.—Artificial-insemination equipment for cattle: *a*, Artificial vagina assembled for use (modification of Swedish model); *b*, semen vials with insulating paper and thumbstalls; *c*, thermometer, 0° to 100° C.; *d*, glass rod, 22 inches long, for applying lubricant to inner tube of the artificial vagina; *e*, rustless-steel inseminator and 2-cc. glass syringe attached; *f*, inseminator constructed of 2-cc. glass pipette connected with 2-cc. glass syringe by short piece of rubber tubing. Note that the apparatus is attached by rubber bands to a celluloid knitting needle to insure rigidity; *g*, wide-mouthed 4-ounce screw-top jar of lubricant. (Courtesy Missouri Agricultural Experiment Station.)

injury to the cow from insertion of the nozzle (or glass tube), and the chances of the cow's breaking the tube by sudden movement are minimized.

COLLECTION BY MEANS OF THE ARTIFICIAL VAGINA

The artificial vagina (figs. 14 and 15) should be filled with water heated to about 54° C. while held in a vertical position. The stopper should then be inserted and the whole inner lining evenly and thoroughly smeared with a thin coat of lubricant. The temperature should be determined by inserting a thermometer in the open end of the artificial vagina. The temperature should not be above 44° nor below 40° when the apparatus is ready for use. If the water is too warm, time should be allowed for cooling; if the water is too cold, some of it should be emptied out and replaced with hot water. Allow-

ance should be made for a slight fall in temperature during the interval that inevitably elapses between the preparation of the apparatus and collection. After the proper temperature is attained the pressure must be adjusted. This may be accomplished by wasting some of the water in the artificial vagina, taking care to wipe the instrument dry thereafter. If the pressure is too great the collecting tube or vial may be forced out when the bull thrusts. About 2 cc. of liquid medicinal paraffin or mineral oil may be placed in the collecting tube or vial before it is inserted if the semen is to be stored for some time.

When the artificial vagina is ready, the bull is led up slowly behind the cow, and the collector follows the bull on the right side, grasping

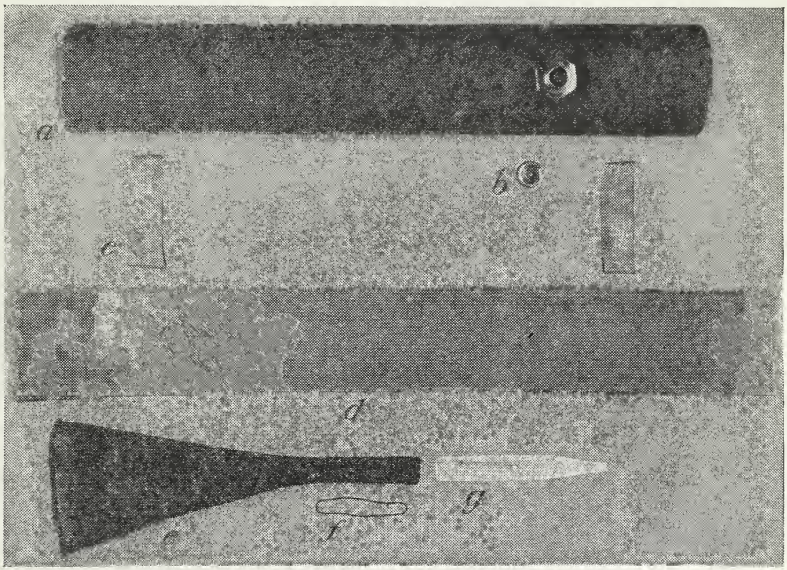

FIGURE 15.—Artificial vagina for cattle, unassembled: *a*, Stiff rubber casing 16 by 2¾ inches with brass valve; *b*, screw cap for valve; *c*, heavy rubber bands for securing each end of the inner tube to the outer casing; *d*, thin rubber tube, 20 inches long by 3 inches flat diameter; *e*, tapering thin rubber tube 10 inches long for connecting artificial vagina; *f*, small rubber band to secure tapering tube to glass tube or vial; *g*, glass collecting tube (8 to 12 cc.). This may or may not be graduated. If one wishes to use mineral oil in the glass collecting tube or vial a straight, flat-bottom vial or ordinary test tube must be used. (Courtesy Missouri Agricultural Experiment Station.)

the apparatus in his right hand, the mouth being held downward. When the bull mounts, the apparatus is inserted behind and to the outside of the bull's foreleg with the opening directed toward the penis at an angle of about 45°. The penis is directed into the opening of the artificial vagina by applying the left hand to the sheath. When the penis comes into contact with the warm lubricated surface of the artificial vagina the bull thrusts upward and ejaculates. Care must be used not to touch the penis as this may cause the bull to retract the penis and dismount. The semen is ejaculated into the upper end of the glass collecting tube or vial of the artificial vagina. Immediately the apparatus should be turned mouth upward to allow all semen to flow into the tube or vial, where it will settle quickly

below the liquid paraffin previously placed therein. Until used, the semen should be kept under paraffin, away from direct sunlight, and at a temperature of 2° to 8° C. If it is to be kept for periods longer than one-half hour, the special procedures that have been described under storage should be followed.

INSEMINATION

The cow should be at the proper stage of estrus and should be confined in a stall or stanchion, preferably with a good light directly behind. In case the light is insufficient, a head lamp may be used (fig. 16). The vulva should be washed clean and dried, as previously described.

The syringe is prepared and filled with the desired quantity of semen. The speculum is held in the left hand with the blades horizontal and to the left and when preparations are complete it is inserted into the vagina by slight pressure forward and upward, rotated so that the handle points down, then opened. The cervix should be visible at the end of the speculum. With the cervix in view, the point of the nozzle is gently inserted from 1 to 2 cm. into the lumen (fig. 6) and from 0.5 to 1.0 cc. of semen expelled slowly into the cervix by gentle pressure on the syringe plunger. It is especially important that only a small volume of semen (0.5 to 1.5 cc.) be used and that it be injected slowly. If volumes in excess of 1.5 cc. are used, inflammation is apt to be set up, and fever and a drop in the milk flow result. The semen should remain in the cervix and not run back into the vagina. In removing the speculum, care should be taken not to pinch the vagina between the blades of the speculum.

When insertion of the speculum causes the cow to arch her back and strain, this can generally be overcome by having the attendant press his knuckles between the cow's vertebrae. If this does not suffice, it may be necessary to remove the speculum and start again. The smaller the diameter of the speculum the less resistance is offered, and hence the straining is less.

Artificial Insemination of Sheep and Goats

The procedures used in the practice of artificial insemination in sheep and goats are similar to those followed for cattle and horses. Before collections or inseminations are undertaken, all apparatus should be carefully cleaned, prepared for use, and laid out on a clean dry table and covered with a clean towel or clean paper. The rams and ewes, if the latter are to be used for collection, should be carefully selected, accustomed to their quarters and to handling, and attention given to those details of management which will facilitate rapid mating with the production of copious ejaculates containing large quantities of semen. The equipment needed, most of which is shown in figure 16 consists of the following:

One 2-cc. glass syringe with glass plunger.

One ebonite or sterling-silver 10-inch nozzle. (10-inch glass tubes of ⅛-inch inner diameter that have been well rounded at one end and slightly tapered and rounded at the other may be substituted for the nozzle. These may be attached to the syringe by short pieces of rubber tubing.)

A semen syringe (a human female urethral-type syringe) may be substituted for the first two items.

Small test tubes or bottles 2 to 5 cc. in size and preferably of Pyrex for holding semen.

A ewe-size speculum. (A Pyrex test tube 6 inches long with an inner diameter of three-fourths of an inch the closed end of which has been cut off makes a very serviceable speculum. The ends must be well rounded in a flame.)

A rubber tube with funnel or kettle with spout for filling artificial vagina with warm water.

An artificial vagina with two spare collecting vials and one spare inner tube.

All collections should be made in the same quarters if possible as a ram quickly learns to anticipate service and will usually mount the ewe more quickly and readily than if in strange quarters. If a ram

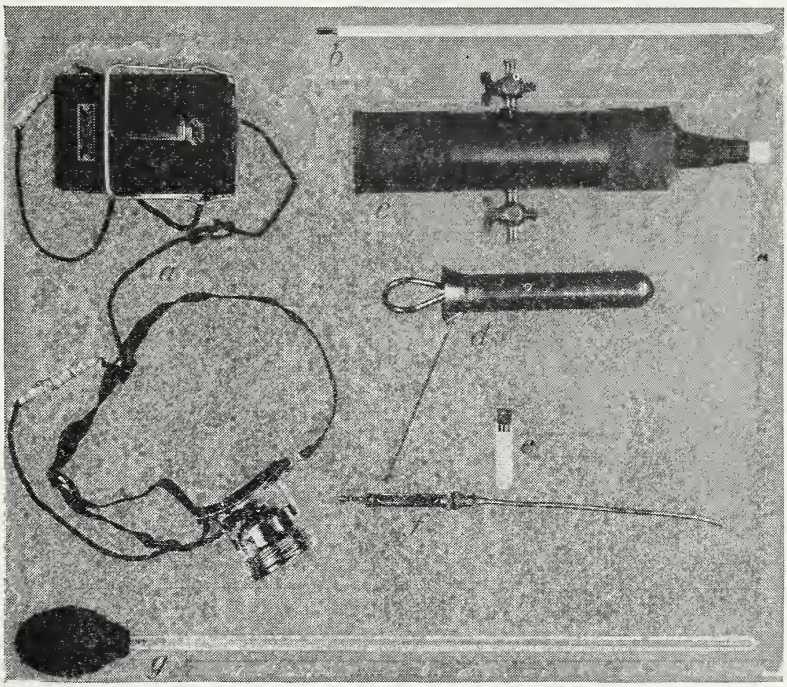

FIGURE 16.—Artificial-insemination equipment for sheep: *a*, Head lamp with battery; *b*, thermometer (0° to 100° C.); *c*, artificial vagina assembled for use (modified from Russian model); *d*, speculum 5 inches by ¾ inch; *e*, glass vial (2-cc.) for storing semen; *f*, inseminator consisting of 2-cc. syringe with glass barrel and 8-inch sterling silver nozzle (human urethral syringe); *g*, semen-collecting syringe for sheep consisting of a glass tube 12 inches long and a rubber bulb. (Courtesy Missouri Agricultural Experiment Station.)

is being used regularly, an interval of one-quarter hour should be allowed between services as a general rule in order to maintain his sexual ardor. When a ram is being trained it is advisable to select ewes that are in heat, and the ram should be allowed to serve a ewe several times before an attempt is made to collect semen. Once a ram is trained, a ewe that is not in heat may be used.

A breeding rack mounted on a stand 18 to 20 inches high facilitates the collection of semen but is not essential (fig. 17). The advantage of using a rack is that it may be set at a convenient level for collecting semen either from the ewe or from the artificial vagina. If a rack is not used, the ewe should be placed in a stanchion, tied to a post or fence, or be held by an assistant.

COLLECTION OF SEMEN FROM THE EWE

A ewe not in heat should be used because the vaginal secretions are not so extensive and, consequently, the semen is obtained in a less contaminated state. Such a ewe may be used repeatedly for collection without any detrimental effects to her. The ram is led up to the ewe and allowed to copulate one to three times, after which he should be removed. The semen is collected from the anterior region of the vagina by means of the syringe (fig. 16) and placed in a test tube or other receptacle. If it is to be retained for any appreciable

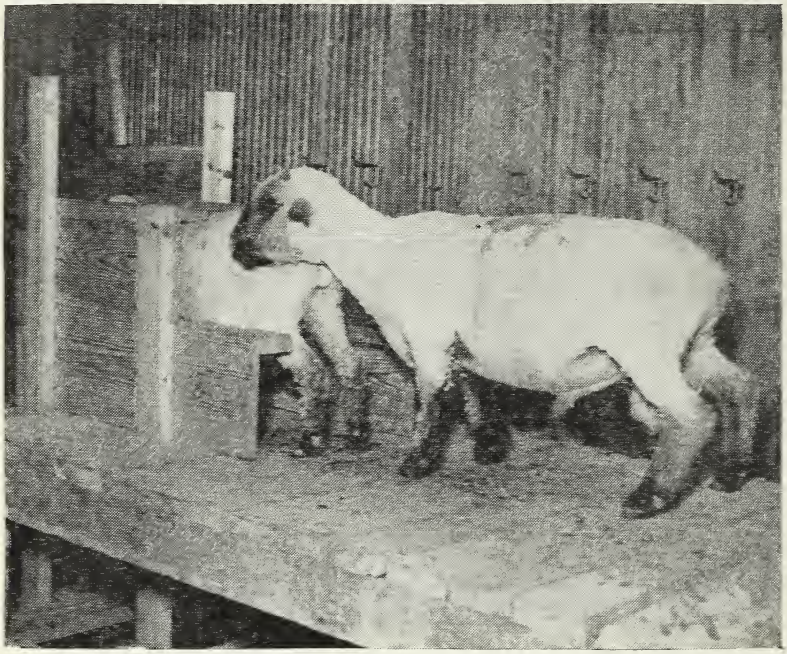

FIGURE 17.—Breeding platform for holding a ewe. The top of the platform should be about 30 inches above the ground. (Courtesy Missouri Agricultural Experiment Station.)

time, it should be placed under paraffin oil and set in a dark place where the temperature is from 2° to 5° C.

COLLECTION BY MEANS OF THE ARTIFICIAL VAGINA

The steps in the preparation of the artificial vagina are similar to those described for similar models used for the bull. The artificial vagina should be partially filled with water that has been heated to 50° C. This may be accomplished by using a rubber tube and funnel or a teakettle. The pressure inside the apparatus is properly adjusted with the right amount of water and air (blown in by mouth). After filling care should be taken to see that the outer surfaces of the apparatus are dry. The surface of the inner tube should then be smeared evenly with a thin coat of lubricant. A thermometer should be inserted in the artificial vagina, the whole apparatus shaken, and the

highest temperature noted, which should be about 45°. Allowance should be made for some fall in temperature between the time of preparation and collection. The temperature of the artificial vagina at the time of collection should be between 41° and 44°. If the temperature drops below 40° or goes above 45° it may inhibit ejaculation. Pressure in the artificial vagina, if too great, may be adjusted by opening a valve, or if too low, by blowing in more air through the valve. An approximation of the desired pressure may be obtained by inserting the thumb into the lumen. It should pass easily, but a slight pressure should be felt. The pressure needed will vary from ram to ram, but with a little experience an operator will soon determine the proper pressure.

When the artificial vagina is ready the operator holds it in his right hand with the open end down at an angle of about 45°, and stands close to the right flank of the ewe. As the ram mounts, the apparatus is interposed behind the ram's foreleg with the opening directed downward toward the penis at an angle of about 45°. The penis is directed into the mouth of the artificial vagina by the left hand, which is applied to the sheath, care being taken not to touch the penis itself. When the penis touches the warm lubricated surface of the artificial vagina, the ram thrusts upward, and semen is ejaculated into the upper end of the tube and collected in the vial. As the ram withdraws, the apparatus is turned mouth upward to permit all of the semen to drain into the vial. The vial is then removed and corked. Subsequent treatment of the semen will depend on whether the semen is to be stored or used immediately.

COLLECTION BY MEANS OF ELECTRICAL STIMULATION

Collection by electrical stimulation, developed by Gunn (*12*), is an effective means of collecting semen from the ram, but, because of the expensive apparatus and the care required in its use, it is valuable chiefly for collections made in the laboratory. The apparatus required (fig. 18) consists of a small transformer of about 2 amperes' capacity, a voltmeter and milliammeter, and two electrodes soldered to insulated wire leads. The alternating current from an electric plant may be rendered suitable by being passed through the primary of a small transformer and tapped in the secondary at voltages ranging from about 10 volts by fives to 40 volts. If an alternating current is not available the current from batteries of suitable voltages may be used, but such current must be passed through a small interrupter and reverser. The intermittent current of reversing polarity is then passed through the voltmeter and milliammeter before being conveyed to the animal. In order that the exact voltage and milliamperage of the current may be determined before it passes to the animal, the leads are passed to a voltmeter and to a milliammeter before continuing to the animal. The electrode for insertion in the rectum consists of a smooth wire of about the diameter of an ordinary lead pencil; the other electrode consists of a wire flattened at one end so that it has a surface area of about one-fourth inch in diameter. Gunn used a stout needle as the second electrode, the needle being inserted in the longissimus dorsi muscle.

In experiments at the United States Sheep Experiment Station an electrode applied tightly to the surface of the skin in the region of the

fourth lumbar vertebra has proved to be just as effective. To insure good contact the skin is first moistened in this region. Both electrodes should be well insulated at the point of soldering to the insulated wire leads in order to prevent shock to the attendant holding the electrodes in place. The apparatus should be placed on a small table about 2 feet high.

When the apparatus is in place and ready for use, the ram should be led in, and laid on his side on a table or bench about 2 feet high, which is a convenient height for working. The ram's forelegs should be tied together and secured in a forward position (fig.19). The hind legs should be similarly secured and extended backwards. The head also should be fixed in an extended position. The feces should first be emptied from the rectum and the long electrode then

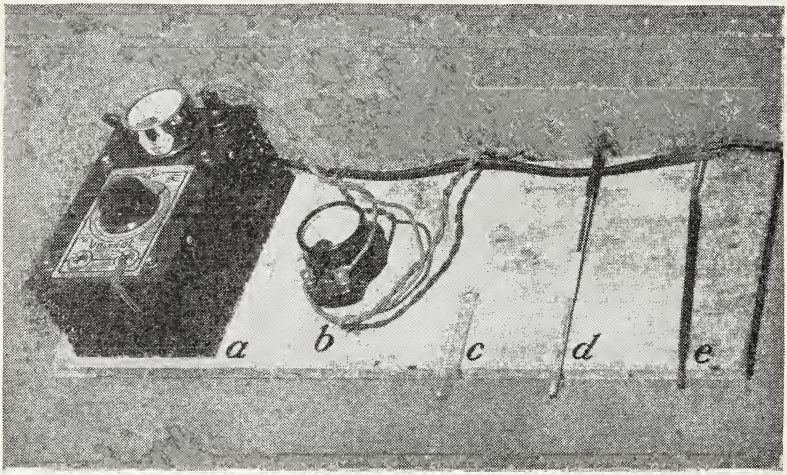

FIGURE 18.—Apparatus and method used for the collection of semen by electrical stimulation at the United States Sheep Experiment Station, Dubois, Idaho. The parts shown are: *a*, Voltmeter; *b*, ammeter; *c*, collecting tube; *d* and *e*, electrodes.

inserted. The other electrode should be firmly held to the skin in the region of the fourth lumbar vertebra by an assistant. A series of 10 to 20 stimulations at 30 volts are then applied, the current being on 5 seconds and off 5 seconds. The number of stimulations given is dependent on the quantity of semen wanted.

When this method is properly used repeated collections may be made from the same ram at daily intervals or on every second day over long periods without any harmful effects. The semen is normal in all respects, but in general the first portion of the ejaculate so obtained is thin and watery in appearance. The main bulk of the ejaculate containing most of the sperm is obtained from the second or third to the twelfth or thirteenth stimulus. Considerable variation is observed between different rams in the number of stimuli required to produce good ejaculation.

The semen is collected in a test tube. Before the collection is made, the penis should be washed with normal saline solution, and if the semen is wanted in a near-sterile condition only the urethral process

should be allowed to enter the neck of the collecting tube. Difficulty is sometimes encountered in securing an extension of the penis, especially in young rams. To accomplish this, the sigmoid flexure should be straightened and the sheath then pushed to the rear. Wrapping a small piece of gauze around the end of the penis helps to keep it extended.

INSEMINATION

Insemination may be carried out most easily if the ewe is placed in a crate which stands 18 to 20 inches off the ground and is mounted on an axle that will allow it to be swung in front of the operator and tilted so as to lower the head of the ewe. In case a crate is not available,

FIGURE 19.—Electrical equipment being used for collection of semen from a ram. Note ammeter and voltmeter, at right.

the ewe may be stood on a table, or a small pit in which the operator can sit may be dug. The purpose is to bring the eyes of the operator on a level with the vulva of the ewe.

The syringe should be filled and made ready for introduction into the vagina. When the ewe is in position the vaginal speculum (fig.16) is introduced into the vagina and the cervix brought into view with the aid of a head lamp. If a glass speculum is used, one end should be rounded somewhat as this facilitates insertion. Before insertion the speculum should be well covered with lubricant. The tip of the nozzle in the syringe is introduced gently by means of the right hand about 1 cm. into the cervix and the plunger depressed until about 0.1 cc. of semen is injected. Volumes of semen in excess of 0.1 or 0.2 cc. are apt to be harmful because of the pain and inflammation sometimes caused. Inseminations should be made during the last half of estrus or at 12-hour intervals so long as estrus lasts.

If a number of ewes are to be inseminated at one time, they should be run into a pen near the place where inseminations are to be made. Careful attention should be given to the details of arrangement of apparatus and equipment to facilitate all operations. If it is necessary to use a dry, dusty place for the purpose the ground should be well sprinkled before either collections or inseminations are undertaken.

Artificial Insemination of Swine

Although artificial insemination has not been practiced to any great extent in swine, enough experimental work has been done to demonstrate that the techniques are quite simple and that the results are satisfactory when inseminations are made at the proper stage of estrus. Semen collections are made by means of an artificial vagina, and the apparatus is only slightly different from the kind used for cattle, horses, and sheep. As the boar produces a large quantity of ejaculate, from 70 to 80 cc. per 100 pounds' weight, according to McKenzie, Miller, and Bauguess (*32*), a large cup is necessary for the semen. Collections may be made with a relatively simple type of vagina in which no special provision is needed to keep the apparatus warm during copulation. Such a device has been described by McKenzie (*30*). It consists of a soft rubber tube (band tubing) 16 inches long and $1\frac{3}{16}$ inches inside diameter and $1\frac{1}{4}$ inches outside diameter, one end of which is fitted over a suction flask and the other end rolled over a $1\frac{5}{8}$-inch key ring. A rubber clamp completes the outfit. More recently Hudjakov (*17*), of the Soviet Union, has described another type of artificial vagina which resembles that used for cattle. It consists of an ebonite cylinder provided with valves for regulating the pressure and an inner rubber chamber. In addition there is a rubber tube 180 mm. long and 80 mm. wide which connects with a semen receptacle of 500 to 800 cc. capacity. Baeckström, of Sweden, uses one similar to that described above for cattle, except that a bulb is attached to an air line to give pulsations.

Another type improvised at the Missouri Agricultural Experiment Station by Lasley and McKenzie consists of an inner tube, $1\frac{5}{16}$ inches in inner diameter and $1\frac{3}{8}$ inches in outer diameter, and an outer rubber casing 12 to 15 inches long (fig. 20, *b*). The semen is collected in any convenient glass receptacle, preferably 50-cc. test tubes.

Collection of Semen by Means of the Artificial Vagina

When the operator is about ready to make the collection the inner surface of the tube is evenly coated with lubricant and the whole apparatus (simple type previously described) immersed in water heated to about 45° C. if the apparatus is to be used in cold weather. Care must be used, however, to avoid getting water into the flask used for collection. A sow is placed in a simple stanchion or tied to a wall by means of a rope around her upper jaw and the boar admitted. When the boar mounts and attempts to copulate, the open end of the tube is placed in front of his sheath so that the penis can pass into the tube. As the penis enters the tube it is manipulated with a pulsating motion by the attendant's hand, thus encouraging continued copulation and ejaculation. Too much pressure must not be applied against the sheath or a part of the contents of the preputial diverticulum may be forced into the collecting flask. The semen is collected in the flask,

and after the boar withdraws, the clamp is placed on the tube just above the flask. From 5 to 20 minutes is required for collection, the time depending upon the condition and disposition of the boar. The Swedish model vagina is used in much the same fashion. Before being used it should be carefully cleaned, the surface of the inner tube evenly smeared with lubricant, and water put in the jacket as in the cattle artificial vagina. A pulsating motion on the penis may be obtained by means of a rubber bulb. The recent Missouri model (fig. 20, *B*) is simple and satisfactory. No water or heat is required, and the correct pressure is adjusted through the air valve prior to use. Pulsations on the penis are made by frequently squeezing the casing. Otherwise the instrument is lubricated and handled as other models.

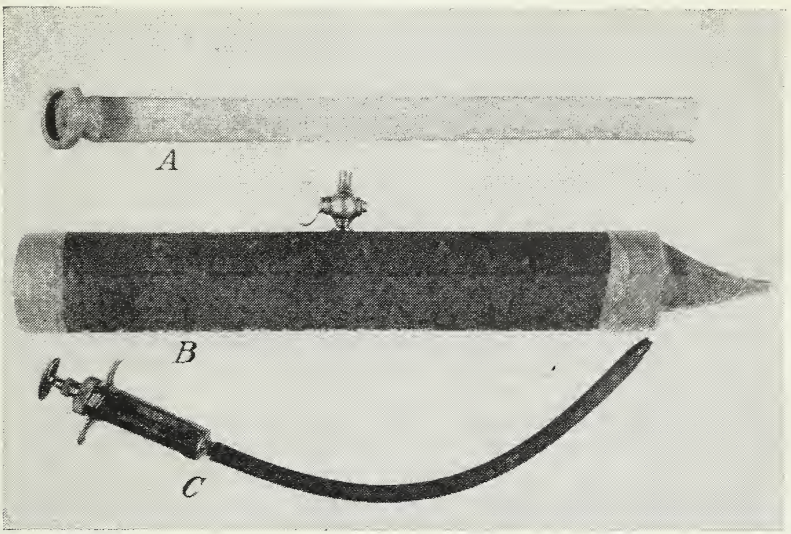

FIGURE 20.—Artificial insemination equipment for swine: *A*, A simple artificial vagina consisting of thin rubber tubing 16 inches long and 1¼ or 1⅜ inches flat diameter with a 1⅝-inch key ring in one end; *B*, another type of artificial vagina consisting of thin rubber tubing similar to the above, together with a rubber casing 12 to 15 inches long and 1¾ inches in diameter. One air valve permits adjustment of air pressure; *C*, inseminator consisting of 50-cc. syringe with glass barrel, and 18-inch rubber pressure tubing, inside diameter 3/16 inch, outside diameter 5/16 inch.

Spermatozoa are not ejaculated uniformly by the boar throughout the period of copulation, according to McKenzie, Miller, and Bauguess (*32*), but appear in waves of great concentration at certain intervals when the rate of discharge of semen is greatest; usually the second to fourth minute of ejaculation. It is well, therefore, to mix the ejaculate well before inseminations are made and to remove the gelatinous lumps. Not more than one service a day should be allowed, and if the boar is being used regularly there should be 1 day of rest following 2 days of service, according to Rodolfo (*55*). This is in general agreement with the findings of McKenzie, Miller, and Bauguess, of the Missouri station (*32*), who noted that with yearlings 2 days of rest between copulations was sufficient to restore both the total volume of semen and the number of spermatozoa, whereas with 1 day of rest the

volume was restored but the sperm number remained somewhat reduced.

Sexual attraction plays relatively little part in the mating behavior of a boar, so it is usually unnecessary to have a sow in heat. Boars, likewise, may be readily stimulated to mount a dummy sow. The quarters used for collection should allow plenty of space for turning and should be equipped with gates to permit easy admission and removal of the animals, and the floors should be of such construction that the danger from slipping is minimized.

INSEMINATION

The structure of the genital tract of the sow is such that only simple apparatus is required for insemination. According to Rodolfo (*56*), the following conditions insure that fertilization will take place following normal mating: (1) The semen is ejaculated directly into the cervix by the corkscrew-shaped glans penis, (2) the volume of semen is very large, and (3) a vaginal plug forms following withdrawal of the penis. The plug is composed of the last fraction of the ejaculate which consists of a stiff waxy material secreted almost entirely by Cowper's glands. In artificial insemination it is desirable that these conditions be simulated. From 50 to 100 cc. of semen should be introduced, and it should be placed well in the anterior end of the vagina. The gelatinous portion of the semen should be discarded before the syringe is loaded. This can be done by separating out these lumps and ejecting them from a glass bowl by a glass rod or by straining the semen through freshly laundered cheesecloth. If the fraction containing the high sperm concentration is used, a smaller volume of only about 25 cc. may be used after it is diluted with a good swine diluter. (See section on Diluters.) After depositing the semen in the anterior end of the vagina of the sow, it is well to push a moistened cotton plug just into the vestibule of the vagina. This prevents excessive waste of semen.

Because of the large uterine horns in the sow, relatively large volumes of semen are sometimes recommended for insemination. No conclusive data are available as to the quantity; but, with undiluted semen, volumes of 50 to 100 cc. seem sufficient for insemination if the sperm concentration in the semen is reasonably high. Using semen diluted four times, Rodin and Lipatov (*54*) state that the optimum volume is 100 to 150 cc. depending on the size of the sow.

Before the sow to be inseminated is led in, all apparatus should be made ready and placed in a convenient location for the operator. The sow should be placed in a crate or breeding chute or tied to a wall by means of a rope around her upper jaw and should, of course, be at the proper stage of estrus (table 2). The equipment needed consists of a glass syringe of 50 cc. capacity, one piece of rubber pressure tubing 45 cm. long and of about 4 mm. inner diameter that has been fitted to the syringe and tapered on the free end (fig. 20). An ebonite nozzle attached to the syringe may be substituted for the rubber tubing. When the sow is ready for insemination and the syringe and tubing (or nozzle) have been filled with semen, the nozzle is introduced into the vagina and forward into the cervix. The semen is slowly expelled into the cervix by pressure on the plunger of the syringe. Hudjakov (17) has described a special apparatus for making injections. It consists of a 600-cc. glass cylinder which is firmly fixed in a wooden

stand. The lower end opens into a rubber tube which is attached to a glass tube in the vagina of the sow. The semen passes into the vagina due to the pressure from the different levels of the fluid and the absorbing capacity of the uterus.

Artificial Insemination of Dogs

Although the dog was one of the first animals used for the study of artificial insemination, relatively little recent experimental work has been done with this species. The techniques of collection and insemination, however, are relatively simple and the method may prove of distinct value to dog breeders.

COLLECTION OF SEMEN

With most dogs collection of the semen is easily made by manual manipulation of the penis. The dog is placed on a table or in a rack at a convenient height from the floor, and the base of the penis is manipulated with the hand until erection is induced. The prepuce is then pushed posterior to the bulbous portion of the penis so that erection is maintained. Under such conditions the accessory glands show irritation and superfunction, ejaculation occurring when the penis is touched. The semen is collected in a clean, dry porcelain or glass receptacle, a 25- to 50-cc. test tube or vial being of convenient size. The semen should then be set in a cool, dark place until it is used. If inseminations are to be made immediately after collection, the semen may be collected directly in the glass syringe used for inseminations by removing the plunger and keeping the finger over the shoulder end of the syringe to prevent escape of the semen. The volume of semen collected will vary with the size and condition of the dog, but for medium-sized dogs the semen will average about 7 cc. Frequent collections may be made without any ill effects, although collections should not be made oftener than once in 2 days if a dog is in regular use for this purpose. Otherwise the sperm count of the semen will be lowered.

Semen may also be collected from the dog by means of an artificial vagina. As early as 1914 Amantea, (*2*), an Italian, used the artificial vagina for the dog. He was apparently the first investigator to use this device for the collection of semen. This method of collection is to be preferred where studies are to be made on physiological problems concerned with ejaculation and sperm production since it simulates normal coitus quite closely. A method for collection of the semen by means of an artificial vagina of the type used for the boar has recently been reported by Alifanow (*1*) of the Soviet Union. His studies revealed that more sperm and a higher quality of sperm were obtained in general from the more aggressive dogs. However, this is not an infallible guide to either quality or quantity of sperm, and a dog should be used extensively for the collection of semen for inseminations only after his semen has been examined carefully under a microscope.

INSEMINATION

Inseminations are made by means of a 20- to 30-cc. glass syringe and a small rubber catheter (cat size) which is attached to the syringe. The female to be inseminated should be in the proper stage of heat,

preferably the eleventh to thirteenth day after beginning to bleed. She should be placed on a table or in a rack and restrained in such a manner as to prevent sudden or excessive movement. The rack should be high enough from the ground to bring the vulva on a level with the eye of the operator. All equipment should be made ready and placed conveniently for the operator before insemination is undertaken.

The vagina should be opened by means of a small speculum; a short test tube of ½-inch inner diameter, the closed end of which has been cut off and the ends well rounded in a flame, does very well. The tip of the catheter should be placed directly in the cervix and the semen slowly expelled by gentle pressure on the plunger of the syringe. The female should be placed so that the vulva is toward a bright light, or preferably the operator should wear a head lamp. This permits direct observation of the cervix during the course of insemination, and the chances of injury to the female are minimized. After the semen is injected, a moist cotton plug should be inserted just inside the vulva, and the hind quarters should be kept elevated for 10 to 15 minutes to prevent loss of the semen as much as possible.

Artificial Insemination of Foxes[16]

Although artificial insemination offers great promise for increasing the use of valuable male foxes, relatively little experimental work has been done with this species. Hence entirely adequate techniques have not been developed, although some measure of success has been obtained with the techniques described below.

COLLECTION OF THE SEMEN

The most satisfactory method that has been devised for the collection of semen from the fox is electrical stimulation.[17] The technique is similar to that employed for sheep. Previous to collection the fox is restrained and laid on his side on a table of convenient working height, about 30 inches, and one electrode is inserted about 3 inches in the rectum. The other electrode is placed between the fourth and fifth lumbar vertebrae. In order that the exact spot for placing the electrode may be located an area approximately 1 inch square should be sheared. As a source of current for these experiments a laboratory magneto mounted in such a way that it could be operated by hand crank, was used. It delivered 35 volts and 20 milliamperes at approximately 30 revolutions per second. The current was applied for 5 seconds and then was cut off for 5 seconds. Ten to twelve shocks were usually sufficient to produce ejaculation. In the early experiments considerable difficulty was experienced due to contamination of the semen with urine, but with improvement of the technique this difficulty was largely obviated.

Copper electrodes 4 to 6 inches in length were used, but the electrode to be inserted into the rectum was covered with glass tubing except for the tip which was flared back over the tubing and then smoothed to prevent injury to the fox. The glass served as an insu-

[16] The authors acknowledge the help of Frank G. Ashbrook and Charles E. Kellogg, of the Bureau of Biological Survey, U. S. Department of the Interior, who have assisted in the preparation of the sections dealing with artificial insemination in foxes and rabbits.

[17] The authors are indebted to R. T. Clark, of Montana State College, Bozeman Mont., who described this method to them in a personal communication.

lator and permitted contact within the fox only at the extreme tip of the electrode.

With this method from ½ to 1 cc. of semen was obtained from a fox at each collection. This is a somewhat smaller volume than was reported by Starkov (*62*), who gives 1.5 cc. as the average volume of the ejaculate obtained from foxes by the method of mechanical manipulation. The range in volume of semen reported by Starkov was from 0.1 to 4.5 cc.

A method of collection by means of mechanical manipulation has been described by Starkov, who states that semen can be easily obtained from the fox by this method. In the hands of workers in the Department of the Interior, however, this method has not proved successful.

INSEMINATION

The technique of insemination used for the bitch is satisfactory also for the vixen. Mating usually takes place toward the end of estrus, namely about 6 days after the beginning of heat, and inseminations should be made, according to Starkov (*62*), at about this time. However, considerably more experimental work is needed on the estrual cycle in the vixen before final recommendations can be made about the optimum time for insemination. Starkov states that the syringe should be retained in the vagina for some time following insemination to prevent wastage of semen. This difficulty could probably be obviated by placing a cotton plug moistened with saline solution just within the vulva.

Adequate methods for storing the semen of foxes have not yet been devised so inseminations should be made as soon as possible after collection.

Artificial Insemination of Rabbits

The rabbit has proved to be an excellent experimental animal for studies on the physiology of reproduction as the doe ovulates only after copulation or after sexual excitement produced by does mounting each other, and at a quite definite interval thereafter, namely, about 8 to 10 hours (fig. 5). As a result this species has been used extensively in an experimental way for studies on artificial insemination and in the Soviet Union to some extent in practical rabbit husbandry, as reported by Padučeva and Maksimov (*46*).

COLLECTION OF SEMEN

Collection by means of an artificial vagina is the most satisfactory method with rabbits. The artificial vagina, devised by Macirone and Walton (*29*), of England, consists of a glass tube or vulcanite cylinder closed at one end by means of a tapering inner rubber sleeve, which is turned back over the edge of the tube and held in place by rubber bands. The rubber sleeve extends through the glass tube and through the center hole of a rubber stopper which closes the other end of the artificial vagina. The semen is collected in a small glass vial inserted through the center hole of the rubber stopper and on the inside of the rubber sleeve (fig. 21, *A*). Water is introduced into the cylinder and the pressure regulated by means of two glass tubes which pass through the rubber stopper and are fitted with rubber connec-

tions. The cylinder is filled with warm water heated to about 45° C., and approximately the right pressure on the rubber sleeve is obtained by holding the tube upright and filling it with water to the level of the tube labeled *g*, in figure 21, *A*. The water is retained in the vagina by clamps on the rubber tubing (*h*). Before being used, the inner surface of the rubber sleeve should be smoothly coated with lubricant.

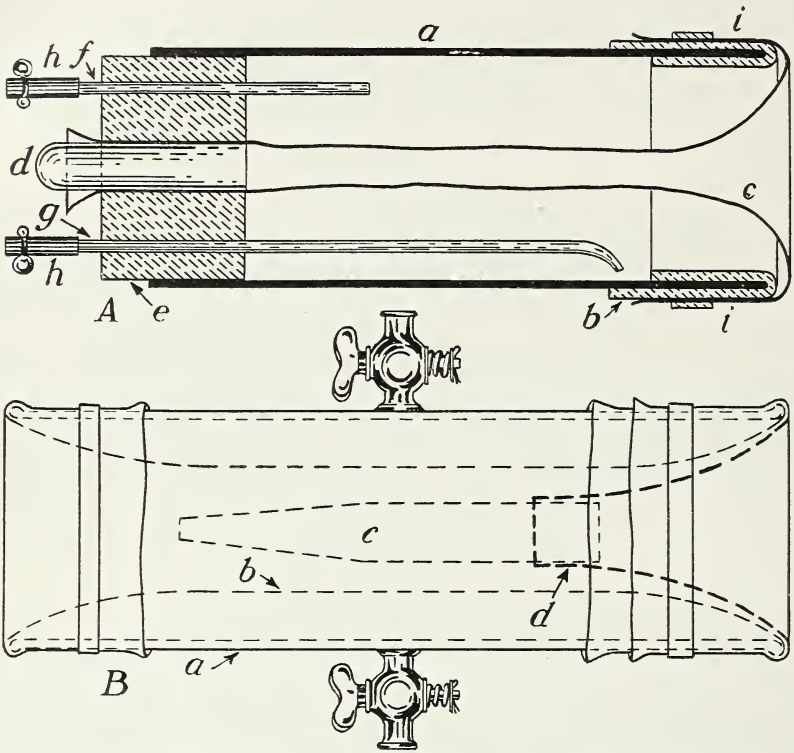

FIGURE 21.—*A*, Longitudinal section of artificial vagina for the rabbit, about actual size: *a*, Glass tube or ebonite cylinder about 1¼ inches in diameter and 3¼ inches long; *b*, thick rubber tubing covering the edge of the cylinder; *c*, thin inner rubber tube or sheath 5 inches long, 1$^{1}/_{16}$ inches in diameter at one end and tapering to ½ inch at the other; *d*, glass specimen tube or vial which fits inside the sheath and holds it in position; *e*, rubber stopper; *f* and *g*, glass tubing for the introduction of water; *h*, rubber connections and clamps; *i*, rubber band to hold the inner sheath in place. (After Macirone and Walton, Journal Agricultural Science, slightly modified.) *B*, Longitudinal section of artificial vagina for rabbit (Missouri model): *a* and *b*, Outer casing and inner tube, respectively, of the sheep artificial vagina; *c*, 10-cc. centrifuge tube; *d*, rubber thumbstall.

Following service the open end of the vagina is turned upward to allow the semen to collect in the container at the inner end of the tube. As with all equipment used for the collection of semen, precautions must be taken to see that the apparatus is clean and dry before it is used, and allowance should be made for a slight drop in the temperature of the water between the time of filling and the time of use. After collection the semen should be set aside in a cool, dark place and handled in a way similar to that described for other species.

Recently Cooksey and McKenzie at the Missouri station have developed an artificial vagina for the rabbit that is simple to make and to operate. The artificial vagina designed for sheep (fig. 16, *c*) has a 10-cc. centrifuge test tube placed in it over the mouth of which a fingerstall is pulled and attached to the cut-off end of the test tube by means of rubber bands. The open end of the fingerstall is stretched and reflected over the end of the sheep artificial vagina. Thus the water jacket of the larger sheep vagina provides adequate heat and pressure, and the fingerstall adapts the instrument to the rabbit (fig. 21, *b*).

This artificial vagina, when ready for use, is held between the hind legs of a doe which has been placed in the buck's cage. When the buck mounts the doe her hind quarters are lifted by the artificial vagina in the operator's hand, and the buck's penis enters the lubricated, open end of the artificial vagina. With a little experience in handling the instrument, collections can be made with any buck that is accustomed to the presence of the operator. Once a buck is accustomed to use the artificial vagina, collections may be made with a dummy. This consists of a glove made of rabbit skin, fur out, which is worn by the operator on the hand in which he holds the artificial vagina.

With the artificial vagina repeated collections may be made from vigorous bucks, and semen in quantities varying from 0.5 to 6.5 cc., in exceptional cases, is obtained. Before the semen is used for artificial insemination, a sample should be checked for sperm concentration, motility, and abnormalities.

Males vary greatly in their sexual desire and, therefore, in the readiness with which they will use the dummy or even copulate with a doe. In general it is best to introduce the female into the cage of the male for most males respond best in familiar surroundings. Once a male becomes accustomed to serving females introduced into his quarters, it is usually easy to substitute the dummy for the female and induce the male to mount.

Semen may be collected also directly from the vagina of the female, after copulation, by aspiration with a small syringe that is inserted into the vagina. If this method is used the doe should be grasped by the hind legs by an assistant and held firmly with the head down. Care must be used in inserting the syringe to avoid injury to the female. The disadvantages of the method are that the yield of semen is small, the semen is mixed with vaginal secretions, and there is the danger of spreading infection.

Another method for the collection of semen consists in inserting in the vagina a small sponge to which a stout thread is fastened. Following copulation, the sponge is removed and immediately squeezed and rinsed in a small quantity of Ringer's solution to remove the semen. The disadvantages of this method are that it is difficult to get good copulation, the yield of semen is small, there is the chance of the spread of infection, and many spermatozoa are injured as they are squeezed from the sponge.

INSEMINATION

Two attendants are required for insemination. When all apparatus has been made ready an assistant grasps the doe and holds her between the knees, head down and with the back toward him, with

the hind legs held uppermost and slightly apart, one in each hand. The second man then inserts the small glass tube used for insemination into the vagina, taking care to pass it over the pelvic brim before squeezing the bulb and expelling the semen. The recommended volume for the doe is from 0.25 to 1.0 cc.

Normally the doe ovulates only after copulation, or sometimes after sexual stimulation due to the mounting of other does, approximately 8 to 10 hours afterward. Consequently if many inseminations are to be made, one or more vasectomized bucks should be on hand. These bucks, if placed with the female, serve the purpose of stimulating ovulation in the doe. The English physiologist, Hammond (*13*), has shown that mating should occur from 5 to 10 hours before ovulation if maximum fertility is to be attained (fig. 5). The drop in both number of fertile matings and the number of young per mating was very rapid if the interval between copulation and ovulation was reduced to less than 5 hours. Since the doe normally ovulates 8 to 10 hours after coitus, insemination should be made within 2 to 5 hours after mating with the vasectomized buck has occurred. If vasectomized bucks are not available, a normal buck may be allowed to mount the doe, but in such a case an apron should be interposed to prevent the insertion of the penis into the vagina. Ordinarily this is sufficient to stimulate ovulation.

Artificial Insemination of the Chicken and Turkey

Although artificial insemination has been practiced but relatively little in birds, recent techniques have been developed that make it useful and practical in certain phases of poultry husbandry. It makes possible a greater use of progeny-tested males, is a valuable means of bringing about fertility of hens kept in batteries, and is valuable in effecting interspecific crosses or in making crosses between breeds that differ greatly in size. And for studies on certain aspects of the physiology of reproduction it is an invaluable tool.

COLLECTION OF SEMEN

A very practicable and easy method for collecting semen from the cock and turkey tom has been described by Burrows and Quinn (*7*), (*8*), (*9*), of the Department of Agriculture. The method, which involves manual manipulation, requires two operators. The bird is held loosely by the thighs by one operator, who supports as much of the bird's weight as possible by extending his fingers under the breast. The rear of the bird is toward the second operator, and the legs of the bird are spread slightly apart so that the abdomen is well exposed. To obtain semen, the second operator causes the copulatory organ to protrude slightly from the vent by rapidly massaging the soft part of the abdomen, while the tail of the bird is forced upward over its back with the heel of the left hand. The thumb and forefinger of the left hand are held in readiness to grasp the vent from above to force the copulatory organ outward as soon as it can be seen protruding from the vent. The semen is expelled into the receiving container, which is held in the right hand of the second operator, by a slow milking manipulation of the copulatory organ. The various procedures involved in collection and insemination are shown in figures 22 and 23.

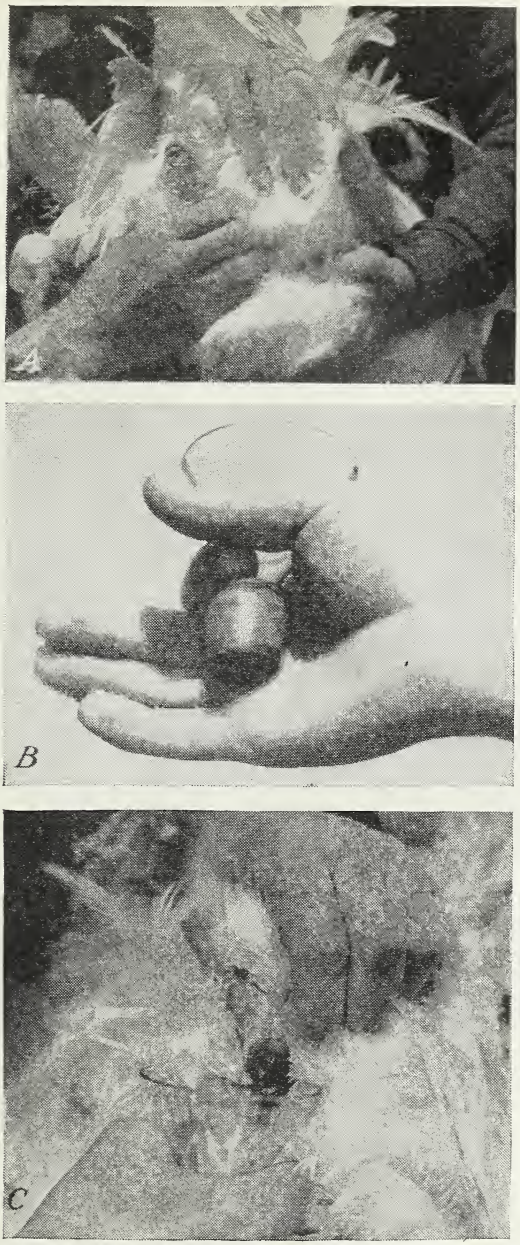

FIGURE 22.—Procedure in the collection of semen from the cock: *A*, Stimulation of the fowl (the operator's left hand is used here only to expose the field for the camera); *B*, container for collecting semen being held so that the fingers are free for stimulation; *C*, showing the left thumb and forefinger holding the copulatory organ exposed. (From Burrows and Quinn.)

With this method of collection it is very important that the male be held loosely, for gripping him tightly inhibits the desired reactions. Continued rapid massage of the abdomen following collection causes the bird to go through an ejaculatory response which assists in refilling the ducts for repeated collections of semen. A bird may be milked from two to six times at each operation, or as long as semen is obtained. It is not necessary to attempt to obtain an ejaculatory response for repeated collections in the turkey. The cock will usually produce

FIGURE 23.—Steps in collecting semen and inseminating the turkey: *A*, Method of holding the tom; *B*, collecting the semen, the copulatory organs exposed; *C*, holding the female for insemination; *D*, method of inseminating the hen.

from 0.2 to 2.0 cc. of semen per day and the tom from 0.1 to 0.8 cc., although the majority of toms, according to Burrows and Marsden (*6*), produce from 0.3 to 0.4 cc. per day. Semen should not be collected oftener than once a day.

A small 60°-angle funnel is used for collection. A one-hole, rubber stopper should be fitted to the funnel to serve as a grip and the stub end below this cut off and the opening filled with paraffin (fig. 22,*B*). If the operation is properly carried out, collection may be made with very little soiling. When contamination with feces occurs, it is obvious because of the discoloration, but contamination from urine may not be so obvious. Where collections are being made from more

than one male, the semen should be transferred, as collected, from the receptacle used for collection to a test tube so that contamination, if it occurs, will ruin but one sample.

Males vary greatly in their response, and some difficulty is usually encountered in making the first collection from a male. In learning the technique it is well to try a number of birds until one is located that responds readily. After the technique is learned on such a bird, collections from other males will be easier. Males kept in batteries or pens apparently produce best. Only an occasional male is found from which semen cannot be obtained by this method.

Parker (*47*) at the Missouri station, has devised a simple technique of collecting semen from the cock. The area surrounding the vent

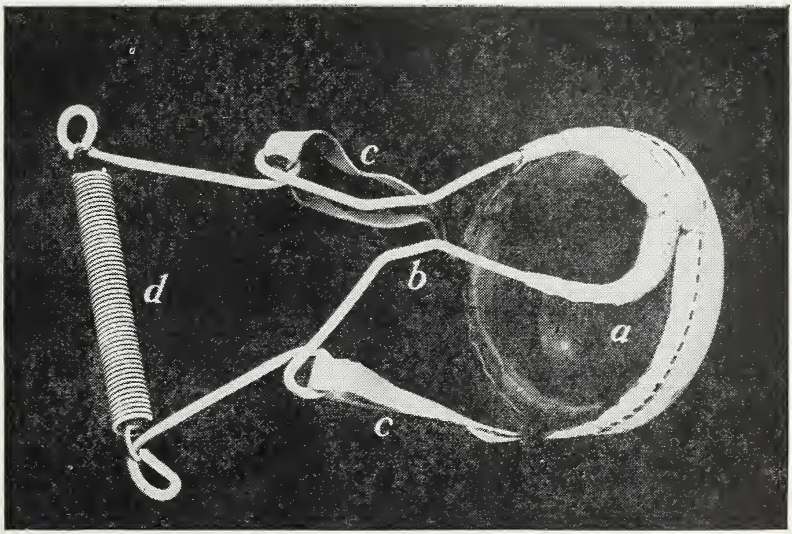

FIGURE 24.—Device for collecting semen from the cock: *a*, Glass receptacle 2½ inches in diameter and 1 inch deep; *b*, wire clamp; *c*, rubber bands; *d*, No. 14 bronze-wire spring. (Courtesy Missouri Agricultural Experiment Station.)

is plucked, wiped with 70-percent alcohol, and a glass cup 2½ inches in diameter and 1 inch deep, which has been coated with paraffin, is held over the vent by means of a clamp over the tail and a No. 14 bronze-wire spring across the back (fig. 24). Elastic bands hold the cup firmly over the vent. The bird is then turned loose among some pullets to tread. He ejaculates into the cup, and the semen is withdrawn from the cup by means of a small pipette or medicine dropper and transferred to a vial. Another model has a removable glass vial attached to the collecting cup, so attached that the semen flows into the glass vial. This type of cup is advantageous if one finds it necessary to collect the semen frequently.

INSEMINATION

The method of inseminating the hen has been described by Quinn and Burrows (*53*) and Burrows and Quinn (*10*). The method consists in exposing the oviduct and injecting semen directly into the

uterus. The hen is held with the left hand under her breast, the index finger between the legs, and the thumb and other fingers around the legs. The loose skin of the abdomen is grasped with the tips of the fingers, which pulls the feathers away from the vent and forces the abdominal contents into the smallest possible space. If properly done, this causes the vent to protrude slightly. The right hand is then placed above the vent so that the thumb and forefinger extend downward on each side of the vent and the hen's tail is forced upward over her back by the heel of the right hand. When the hands are in this position a sudden pressure between them will cause the oviduct to be everted as in normal mating. The eversion will be easier if the whole procedure is done quickly after the hen is picked up.

When the oviduct is sufficiently everted so that its orifice can be plainly seen, the inseminating syringe is introduced as far as it will slide easily, usually about 2 inches. Pressure on the abdomen is then fully released, while slight pressure is maintained on the syringe, which causes it to follow the retraction of the oviduct. When the oviduct is fully retracted about one-third of the syringe should be covered. At this point the desired amount of semen is injected by gentle pressure on the plunger of the syringe. A common 1-cc. tuberculin-type syringe, graduated in 0.1-cc. units is most satisfactory for this purpose. All equipment should be made ready and the syringe filled with the requisite amount of semen before any inseminating is undertaken.

On account of its size the turkey hen is handled somewhat differently. The operator picks the hen up with her head toward him, then stoops slightly and thrusts her head between his legs, which permits the bird to rest on his sloping legs (fig. 23, C). The legs are held in the right hand until the left hand is in position on the abdomen. From this point the procedure is similar to that followed with the chicken hen. To bring about eversion of the oviduct, considerable pressure must sometimes be applied; but, if it is applied quickly, eversion will be brought about with less pressure than if the pressure is applied slowly.

Using this method of insemination, Burrows and Marsden (*6*) obtained 80-percent fertility on 31 turkey hens inseminated with 0.05 cc. of turkey semen at intervals of 1, 2, 3, and 4 weeks. These observations were based on a total of 948 eggs. In one special test mating of 10 turkey hens with an old tom the percentage fertility was increased from 7.5, for natural mating, to 88.4, when artificial insemination was used. No greater embryonic mortality was observed in eggs from hens artificially inseminated than in eggs from hens naturally mated. Hens that become fertile may retain their fertility for 3 to 4 weeks, but all hens do not become fertile from a single insemination. If hens are properly inseminated with good viable semen twice at an interval of 3 to 4 days with 0.5 cc. of semen and this process is repeated every 3 weeks, from 80- to 90-percent fertility should be obtained, although the degree of fertility will vary with different hens.

In chickens insemination with 0.1 cc. of good semen once each week should result in 80- to 90-percent fertility of the eggs, although this may vary somewhat from mating to mating. If the fertility for any mating under such procedure is low, it may be necessary to use more semen, to inseminate more frequently, or both. Ordinarily the best fertility is obtained when inseminations are made with semen that

has just been collected. Satisfactory methods have not yet been developed for storing semen more than a few hours.

As in normal mating, some hens, both chickens and turkeys, do not become fertile after careful and persistent inseminations. Such hens should not be kept in the breeding flock.

Use of Dummies for the Collection of Semen From Livestock

If the artificial vagina is used for the collection of semen and frequent collections are to be made, the use of dummies is practicable. The use of a dummy eliminates the necessity for having females in heat and also the extra work involved in having females on hand for collection.

The males of most species may be readily trained to use a dummy. They have been used successfully with stallions, bulls, boars, rams, and rabbits, although their use is not to be recommended unless a large number of collections are to be made with the artificial vagina. In training the male to use a dummy he should first be used to serve females that are in heat, and the same quarters should be used for this purpose each time. As a result he soon learns to anticipate service when brought into these quarters, and after he has become accustomed to expecting service the dummy may be substituted for the female. Not all males train with the same ease, and with certain species more difficulty is encountered than with others. Young, vigorous males as a rule may be most readily trained to use the dummy. Sight or odor apparently plays a relatively small part in the mating instinct of the male in most species although some variability in this respect exists between individual males as well as between males of different species.

The dummy should be solidly constructed and firmly anchored and should resemble somewhat, although not necessarily, the female of the species for which it is being used (fig. 7). The framework may be of either metal or wood, the only precaution necessary being that it be adequate to support the weight of any males used for collection. The top and sides of the dummy should be well padded and the structure covered with a skin, with canvas, or with some other durable material. Space may be provided for the insertion of the artificial vagina. The latter may be held in place with straps, but in the larger models it is better if it can be held by an assistant, who should be seated under the dummy.

Use of Diluters

The primary purpose of diluters is to increase the volume of the ejaculate of a male so that it may be used to inseminate a larger number of females. In the Soviet Union diluters have been used extensively for this purpose at the artificial-insemination centers, with very good results. For certain purposes, as in breeding associations where a sire is used for large numbers of females, the use of diluters is essential.

A good diluter should have the following qualities: (1) It must not be toxic to spermatozoa, (2) the osmotic relations must be similar

to those present in the undiluted semen, (3) the hydrogen-ion concentration of the solution must be favorable for continued viability of the spermatozoa, (4) it should contain a buffering solution to protect against marked changes in the hydrogen-ion concentration, and (5) the diluter should be inexpensive and easy to prepare. A Russian investigator, Milovanov (*37*), has described diluting solutions which apparently meet these requirements satisfactorily. Formulas for diluters developed by Milovanov (*38*) for those species in which artificial insemination has been used most extensively are listed in tables 4 and 5.

TABLE 4.—*Formulas for tartrate diluters for semen of farm animals*

Animal	Glass-distilled water	Anhydrous glucose ($C_6H_{12}O_6$)	Sodium potassium tartrate ($KNaC_4H_4O_6$)	Peptone (salt-free)
	Grams	*Grams*	*Grams*	*Grams*
Stallion	1,000	57.0	6.7	2.0
Bull	1,000	12.0	25.6	5.0
Boar	1,000	46.1	5.6	3.5
Buck (rabbit)	1,000	39.0	7.0	2.0

TABLE 5.—*Formulas for sulfate and phosphate diluters for semen of farm animals*

Animal	Glass-distilled water	Anhydrous sodium sulfate (Na_2SO_4)	Anhydrous glucose ($C_6H_{12}O_6$)	Peptone (salt-free)	Disodium phosphate Na_2HPO_4 ($12H_2O$)	Monopotassium phosphate (KH_2PO_4)	Calcium lactate ($CaC_6H_{10}O_6$ $3H_2O$)
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Stallion	1,000	3.4	57.6	2.0			
Bull	1,000	13.6	12.0	5.0			
Ram	1,000		50.4		6.78	0.15	1.91
Boar	1,000	2.8	46.1	3.5			
Buck (rabbit)	1,000	3.6	39.0	2.0			

The phosphoric acid-calcium sediment which is precipitated, in the case of the sheep-semen diluter, is filtered off. The optimum dilutions for these solutions, as reported by Milovanov (*37*), are respectively in parts of semen to diluter: Stallion, 1 to 7; bull, 1 to 15; ram, 1 to 31; boar, according to Rodin and Lipatov (*54*), 1 to 4; and buck rabbits, 1 to 15.

Formulas for other diluters are given by Winters et al. (*74*), Sorensen (*61*) and Küst (*25*). Küst and the Missouri station workers have had much success with 0.9-percent sodium chloride in bull semen and 0.8-percent for ram semen.[18]

Gunn (*12*) has tried the effects of various fluids on the longevity of ram sperm as measured by duration of motility. The substances tested were neutral glucose saline, sterile blood serum of the horse and sheep, secretions collected from the vagina of ewes in estrus, alkaline normal saline containing 0.89 percent of sodium chloride and 0.10 percent of sodium bicarbonate, acetylcholine, and thin sterile starch. In only two of the solutions, the alkaline normal saline and starch solutions, was there any marked increase in longevity, and in the latter solution the vigor of the sperms was somewhat decreased.

A Canadian investigator, Munro (*43*), has found that motility of fowl sperm in vitro depends upon an interplay of temperature and

[18] McKenzie and Steensma, unpublished data.

the medium of suspension. Most synthetic diluents will support motility at room temperature but inhibit it at body temperature. Movement is again resumed, however, when the temperature is lowered. Rat and guinea-pig sperm on the other hand, are immobilized by low temperatures and are most active at body temperatures in the same diluents. Among natural fluids, serum from semen, blood serum, thin egg white, and fluid from the shell gland were found to support the motility of fowl sperm at all levels from 75° to 105° F., whereas fluid from the magnum or the infundibulum behaved as synthetic diluents, immobilizing sperm at 105° but supporting motility at lower temperatures.

Walton (*66*) states that if diluters are used they should be added to the semen slowly, drop by drop, the semen being shaken while the diluter is added.

Baker (*4*), an English biologist, has described certain substances which have the property of stimulating spermatozoa at certain concentrations. Thus, it was found that strychnine hydrochloride stimulated guinea-pig sperm strongly in concentrations of 0.16 and 0.64 percent and less strongly at concentrations of 0.256 percent. Sperm so stimulated were not damaged and gave rise to normal offspring. McKenzie and Steensma[19] have found that this drug has a decidedly stimulating effect on ram and bull semen stored for 3 days. Although further experimental work is needed before conclusions can be drawn, it appears that this and other solutions may be successfully used to bring about fertility of valuable males where sterility is due to a feeble condition of the spermatozoa, and it seems to offer promise as a means of restoring vigor to spermatozoa that have lost their vitality as a result of storage.

Recently there has been developed a very satisfactory diluter for cattle at the University of Wisconsin.[20] This diluter is made up as follows:

To 100 cc. of boiling distilled water add—

0.2 gram KH_2PO_4(chemically pure).

2.0 grams $Na_2HPO_4.12H_2O$(chemically pure).

After this mixture has cooled to room temperature add an equal volume of fresh egg yolk which has been carefully separated from the whites. To one part of bull semen slowly add three parts of this egg-yolk mixture.

For horse and jack semen the Missouri station recommends the use of the same egg-yolk buffer except that to the above 100 cc. of boiling distilled water 10 grams of dextrose or glucose (chemically pure) are added. One part of horse or jack semen is diluted with one part of this glucose and egg-yolk buffer. This dilution is done immediately after collection and before refrigeration.

[19] Unpublished data, Missouri station.

[20] Lardy, Henry A., and Paul H. Phillips, Preservation of Spermatozoa. Amer. Soc. Anim. Prod. Proc. 219-221, 1939.

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